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=> d his

     (FILE 'HOME' ENTERED AT 09:44:07 ON 21 SEP 2004)
               SET COST OFF

     FILE 'REGISTRY' ENTERED AT 09:44:26 ON 21 SEP 2004
               E SMURF
L1           20 S E3-E5 OR ?SMURF?/CNS
               E SMAD
L2          403 S E3-E21

     FILE 'HCAPLUS' ENTERED AT 09:46:07 ON 21 SEP 2004
L3           15 S L1
L4           85 S ?SMURF?
L5           90 S L3,L4
L6            9 S L5 AND L2
L7           48 S L5 AND ?SMAD?
L8           50 S L6,L7
L9           42 S L8 AND UBIQUITIN?
L10          50 S L8,L9
L11           7 S L10 AND SCREEN?
               E DRUG SCREENING/CT
L12       24987 S E3-E5
L13        6373 S E9,E10
               E E3+ALL
L14       31124 S E9,E8
               E E12+ALL
L15        9001 S E10
L16        3897 S E21+NT
L17           5 S L10 AND L12-L16
L18           7 S L11,L17
L19           8 S L10 AND ?MODULAT?
L20          11 S L5 AND ?MODULAT?
L21          15 S L18-L20
L22           0 S L10 AND ?PPYX?
L23           7 S L10 AND WW (L) DOMAIN
L24          21 S L21,L23
               E WRANA J/AU
L25         117 S E3-E9
               E THOMSEN G/AU
L26          35 S E3-E6
L27           9 S L25,L26 AND L5
L28          25 S L24,L27
L29          18 S L5 AND (PD<=19990611 OR PRD<=19990611 OR AD<=19990611)
L30           2 S L28 AND L29
L31           0 S L5 AND (PY<=1999 OR PRY<=1999 OR AY<=1999) NOT L29
L32           9 S L27,L30
L33          16 S L29 NOT L32
               SEL DN AN 2-9 11-16
L34           2 S L33 NOT E1-E38
L35          11 S L32,L34
L36           3 S L3 AND L29
L37          11 S L35,L36

=> fil hcaplus
FILE 'HCAPLUS' ENTERED AT 09:57:27 ON 21 SEP 2004
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FILE COVERS 1907 - 21 Sep 2004 VOL 141 ISS 13
FILE LAST UPDATED: 20 Sep 2004 (20040920/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> d l37 all tot

L37 ANSWER 1 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:941411 HCAPLUS
DN 140:3090
ED Entered STN: 03 Dec 2003
TI Regulation of Cell Polarity and Protrusion Formation by Targeting RhoA for Degradation
AU Wang, Hong-Rui; Zhang, Yue; Ozdamar, Barish; Ogunjimi, Abiodun A.; Alexandrova, Evguenia; **Thomsen, Gerald H.**; **Wrana, Jeffrey L.**
CS Samuel Lunenfeld Research Institute, Program in Molecular Biology and Cancer, Mount Sinai Hospital, Toronto, M56 1X5, Can.
SO Science (Washington, DC, United States) (2003), 302(5651), 1775-1779
CODEN: SCIEAS; ISSN: 0036-8075
PB American Association for the Advancement of Science
DT Journal
LA English
CC 13-2 (Mammalian Biochemistry)
AB The Rho family of small guanosine triphosphatases regulates actin cytoskeleton dynamics that underlie cellular functions such as cell shape changes, migration, and polarity. We found that **Smurf1**, a HECT domain E3 ubiquitin ligase, regulated cell polarity and protrusive activity and was required to maintain the transformed morphol. and motility of a tumor cell. Atypical protein kinase C zeta (PKCζ), an effector of the Cdc42/Rac1-PAR6 polarity complex, recruited **Smurf1** to cellular protrusions, where it controlled the local level of RhoA. **Smurf1** thus links the polarity complex to degradation of RhoA in lamellipodia and filopodia to prevent RhoA signaling during dynamic membrane movements.
ST **Smurf1** C kinase actin RhoA degrdn cell polarity morphol
IT Rho protein (G protein)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(RhoA; regulation of cell polarity and protrusion formation by targeting RhoA for degradation)
IT Cell morphology
Cytoskeleton
(regulation of actin cytoskeleton dynamics and cell polarity and protrusion formation by targeting RhoA for degradation)
IT Actins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(regulation of actin cytoskeleton dynamics and cell polarity and protrusion formation by targeting RhoA for degradation)
IT Protein degradation
(regulation of cell polarity and protrusion formation by targeting RhoA for degradation)
IT 74812-49-0, E3 Ubiquitin ligase
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(**Smurf1**; protein kinase Cζ recruits **Smurf1** to cellular protrusions, where it controls local level of RhoA)
IT 472998-88-2, Protein kinase Cζ
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein kinase Cζ recruits **Smurf1** to cellular protrusions, where it controls local level of RhoA)
RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Anon; www.sciencemag.org/cgi/content/full/302/5651/1775/DC1
(2) Bar-Sagi, D; Cell 2000, V103, P227 HCAPLUS
(3) Betschinger, J; Nature 2003, V422, P326 HCAPLUS
(4) Bishop, A; Biochem J 2000, V348, P241 HCAPLUS
(5) Bonni, S; Nature Cell Biol 2001, V3, P587 HCAPLUS
(6) Coghlan, M; Mol Cell Biol 2000, V20, P2880 HCAPLUS
(7) Etienne-Manneville, S; Cell 2001, V106, P489 HCAPLUS
(8) Etienne-Manneville, S; Nature 2002, V420, P629 HCAPLUS
(9) Etienne-Manneville, S; Nature 2003, V421, P753 HCAPLUS
(10) Gao, L; Curr Biol 2002, V12, P221 HCAPLUS
(11) Gomes, J; Curr Biol 2002, V12, PR444 HCAPLUS
(12) Hall, A; Br J Cancer 1999, V80(suppl 1), P25
(13) Hall, A; Philos Trans R Soc London B Biol Sci 2000, V355, P965 HCAPLUS
(14) Harvey, K; Trends Cell Biol 1999, V9, P166 HCAPLUS
(15) Hershko, A; Annu Rev Biochem 1998, V67, P425 HCAPLUS
(16) Hung, T; Development 1999, V126, P127 HCAPLUS
(17) Joberty, G; Nature Cell Biol 2000, V2, P531 HCAPLUS
(18) Kaibuchi, K; Annu Rev Biochem 1999, V68, P459 HCAPLUS
(19) Kavsak, P; Mol Cell 2000, V6, P1365 HCAPLUS
(20) Levy, F; Proc Natl Acad Sci USA 1996, V93, P4907 HCAPLUS
(21) Lin, D; Nature Cell Biol 2000, V2, P540 HCAPLUS
(22) Lin, X; J Biol Chem 2000, V275, P36818 HCAPLUS
(23) Nobes, C; J Cell Biol 1999, V144, P1235 HCAPLUS
(24) Plant, P; Nature Cell Biol 2003, V5, P301 HCAPLUS
(25) Qiu, R; Curr Biol 2000, V10, P697 HCAPLUS
(26) Qiu, R; Mol Cell Biol 1997, V17, P3449 HCAPLUS
(27) Schmidt, A; Genes Dev 2002, V16, P1587 HCAPLUS
(28) Shi, S; Cell 2003, V112, P63 HCAPLUS
(29) Suzuki, A; J Cell Biol 2001, V152, P1183 HCAPLUS
(30) Suzuki, A; J Cell Sci 2002, V115, P3565 HCAPLUS
(31) Tabuse, Y; Development 1998, V125, P3607 HCAPLUS
(32) Van Aelst, L; Genes Dev 2002, V16, P1032 HCAPLUS
(33) Wang, H; unpublished data
(34) Zhang, Y; Proc Natl Acad Sci USA 2001, V98, P974 HCAPLUS
(35) Zhu, H; Nature 1999, V400, P687 HCAPLUS

L37 ANSWER 2 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:813733 HCAPLUS
DN 140:126177
ED Entered STN: 16 Oct 2003
TI The RING-H2 protein RNF11 is overexpressed in breast cancer and is a target of **Smurf2** E3 ligase
AU Subramaniam, V.; Li, H.; Wong, M.; Kitching, R.; Attisano, L.; **Wrana, J.**; Zubovits, J.; Burger, A. M.; Seth, A.
CS CIHR Group in Matrix Dynamics, Sunnybrook and Women's College Health Sciences Centre, 1Laboratory of Molecular Pathology and Molecular and Cellular Biology Research, University of Toronto, Toronto, ON, Can.
SO British Journal of Cancer (2003), 89(8), 1538-1544
CODEN: BJCAAI; ISSN: 0007-0920
PB Nature Publishing Group
DT Journal
LA English
CC 14-1 (Mammalian Pathological Biochemistry)
AB The breast cancer-associated T2A10 clone was originally isolated from a cDNA library enriched for tumor messenger ribonucleic acids. Our survey of 125

microarrayed primary tumor tissues using affinity purified polyclonal antibodies has revealed that corresponding protein is overexpressed in invasive breast cancer and is weakly expressed in kidney and prostate tumors. Now known as RNF11, the gene encodes a RING-H2 domain and a PY motif, both of which mediate protein-protein interactions. In particular, the PPPPY sequence of RNF11 PY motif is identical to that of Smad7, which has been shown to bind to WW domains of **Smurf2**, an E3 ubiquitin ligase that mediates the ubiquitination and degradation of the TGFβ receptor complex. Using various mutants of RNF11 in GST pulldown and immunopptn. assays, we found that RNF11 interacts with **Smurf2** through the PY motif, leading to ubiquitination of both proteins. **Smurf2** plays an active role in the repression of TGFβ signaling, and our data indicate that overexpression of RNF11, through its interaction with **Smurf2**, can restore TGFβ responsiveness in transfected cells.

ST **Smurf2** RNF11 protein breast cancer

IT Protein motifs
(PY motif, of RNF11; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Bladder, neoplasm
Head, neoplasm
Lung, neoplasm
(RING-H2 protein RNF11 expression in)

IT Human
Mammary gland, neoplasm
Molecular association
Signal transduction, biological
(RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(RNF11; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Protein motifs
(WW domains, of **Smurf2**; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Pancreas, neoplasm
(adenocarcinoma; RING-H2 protein RNF11 expression in)

IT Prostate gland, neoplasm
(carcinoma; RING-H2 protein RNF11 expression in)

IT Intestine, neoplasm
(colon; RING-H2 protein RNF11 expression in)

IT Neck, anatomical
(neoplasm; RING-H2 protein RNF11 expression in)

IT Kidney, neoplasm
(renal cell carcinoma; RING-H2 protein RNF11 expression in)

IT Enzymes, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ubiquitin-conjugating, UBCH5A; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Transforming growth factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(β-; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Transforming growth factor receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(β-transforming growth factor; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT 74812-49-0, E3 Ubiquitin ligase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(RING-H2 protein RNF11 is overexpressed in breast cancer and is target

of **Smurf2** E3 ligase)
IT 60267-61-0, Ubiquitin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ubiquitination; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)
RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Attisano, L; Science 2002, V296, P1646 HCAPLUS
(2) Bonni, S; Nat Cell Biol 2001, V3, P587 HCAPLUS
(3) Burger, A; Oncogene 1998, V16, P327 HCAPLUS
(4) Chen, A; J Biol Chem 2000, V275, P15432 HCAPLUS
(5) Conaway, R; Science 2002, V296, P1254 HCAPLUS
(6) Coopman, P; Nature 2000, V406, P742 HCAPLUS
(7) Di Guglielmo, G; Nat Cell Biol 2003, V5, P410 HCAPLUS
(8) Freemont, P; Curr Biol 2000, V10, PR84 HCAPLUS
(9) Fukuchi, M; Cancer Res 2002, V62, P7162 HCAPLUS
(10) Hayashi, H; Cell 1997, V89, P1165 HCAPLUS
(11) Hershko, A; Annu Rev Biochem 1998, V67, P425 HCAPLUS
(12) Hoodless, P; Cell 1996, V85, P489 HCAPLUS
(13) Jackson, P; Trends Cell Biol 2000, V10, P429 HCAPLUS
(14) Joazeiro, C; Cell 2000, V102, P549 HCAPLUS
(15) Kavsak, P; Mol Cell 2000, V6, P1365 HCAPLUS
(16) Kitching, R; Biochim Biophys Acta, in press 2003
(17) Kononen, J; Nat Med 1998, V4, P844 HCAPLUS
(18) Landberg, G; Oncogene 2001, V20, P3497 HCAPLUS
(19) Li, H; An RNF11:Smurf2 complex mediates ubiquitination of the AMSH protein, in review process 2003
(20) Longnecker, R; Exp Cell Res 2000, V257, P332 HCAPLUS
(21) Maeda, I; FEBS Lett 2001, V494, P181 HCAPLUS
(22) Mehra, A; Biochem Cell Biol 2002, V80, P605 HCAPLUS
(23) Mizuide, M; J Biol Chem 2003, V278, P531 HCAPLUS
(24) Seki, N; Biochim Biophys Acta 1999, V1489, P421 HCAPLUS
(25) Suzuki, C; J Biol Chem 2002, V277, P39919 HCAPLUS
(26) Thrower, J; EMBO J 2000, V19, P94 HCAPLUS
(27) Winberg, G; Mol Cell Biol 2000, V20, P8526 HCAPLUS
(28) Yagi, R; EMBO J 1999, V18, P2551 HCAPLUS
(29) Yendamuri, S; Cancer Res 2003, V63, P878 HCAPLUS
(30) Zheng, N; Nature 2002, V416, P703 HCAPLUS

L37 ANSWER 3 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:493891 HCAPLUS
DN 140:315556
ED Entered STN: 30 Jun 2003
TI Distinct endocytic pathways regulate TGF-β receptor signalling and turnover [Erratum to document cited in CA139:191931]
AU Di guglielmo, Gianni M.; Le Roy, Christine; Goodfellow, Anne F.; **Wrana, Jeffrey L.**
CS Samuel Lunenfeld Researh Institute, Programme in Molecular Biology and Cancer, Mount Sinai Hospital, Toronto, ON, Can.
SO Nature Cell Biology (2003), 5(7), 680
CODEN: NCBIFN; ISSN: 1465-7392
PB Nature Publishing Group
DT Journal
LA English
CC 2-10 (Mammalian Hormones)
AB On page 414, second column, line 9, the text should read "Autoradiog. (Fig. 1g) and quantitation (Fig. 1h)..." rather than "Autoradiog. (Fig. 1g) and quantitation (Fig. 1g)...".
ST erratum TGFbeta receptor endocytosis endosome vesicle lipid caveolae signaling; endocytosis TGF beta receptor signaling turnover erratum
IT Clathrin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(-dependent endocytosis; distinct endocytic pathways regulate

TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Lipids, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study) (-rafts on cell membrane; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Antigens
RL: BSU (Biological study, unclassified); BIOL (Biological study) (EEA-1 (early endosome antigen-1); distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study) (SARA (Smad anchor for receptor activation); distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study) (Smad-2; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study) (Smad-7; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study) (**Smurf2**; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Transforming growth factor receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study) (TGF-β receptor, type I; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Transforming growth factor receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study) (TGF-β receptor, type II; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Organelle
(caveolae; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study) (caveolins; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Organelle
(coated pit; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Endocytosis
Endosome
Human
Protein degradation
Signal transduction, biological
(distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Organelle
(endocytic vesicle, early; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Biological transport
(internalization; distinct endocytic pathways regulate TGF-β

receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Cell membrane
(lipid rafts on-; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Phosphorylation, biological
(protein; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT Transforming growth factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(β-; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT 74812-49-0
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**Smurf2**; distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))
IT 60267-61-0, Ubiquitin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(distinct endocytic pathways regulate TGF-β receptor signaling and turnover as studied in mammalian cells (Erratum))

L37 ANSWER 4 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:332595 HCAPLUS
DN 139:191931
ED Entered STN: 01 May 2003
TI Distinct endocytic pathways regulate TGF-β receptor signalling and turnover
AU Di Guglielmo, Gianni M.; Le Roy, Christine; Goodfellow, Anne F.; **Wrana, Jeffrey L.**
CS Samuel Lunenfeld Research Institute, Programme in Molecular Biology and Cancer, Mount Sinai Hospital, Toronto, Can.
SO Nature Cell Biology (2003), 5(5), 410-421
CODEN: NCBIFN; ISSN: 1465-7392
PB Nature Publishing Group
DT Journal
LA English
CC 2-10 (Mammalian Hormones)
AB Endocytosis of cell surface receptors is an important regulatory event in signal transduction. The transforming growth factor β (TGF-β) superfamily signals to the Smad pathway through heteromeric Ser-Thr kinase receptors that are rapidly internalized and then downregulated in a ubiquitin-dependent manner. TGF-β receptors internalize into both caveolin- and EEA1-pos. vesicles and reside in both lipid raft and non-raft membrane domains. Clathrin-dependent internalization into the EEA1-pos. endosome, where the Smad2 anchor SARA is enriched, promotes TGF-β signaling. In contrast, the lipid raft-caveolar internalization pathway contains the Smad7-**Smurf2** bound receptor and is required for rapid receptor turnover. Thus, segregation of TGF-β receptors into distinct endocytic compartments regulates Smad activation and receptor turnover.
ST TGF beta receptor endocytosis endosome vesicle lipid caveolae signaling
IT Clathrin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(-dependent endocytosis; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Lipids, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(-rafts on cell membrane; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)

IT Antigens
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(EEA-1 (early endosome antigen-1); distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(SARA (Smad anchor for receptor activation); distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Smad-2; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Transcription factors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Smad-7; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**Smurf2**; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Transforming growth factor receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TGF-β receptor, type I; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Transforming growth factor receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(TGF-β receptor, type II; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Organelle
(caveolae; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(caveolins; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Organelle
(coated pit; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Endocytosis
Endosome
Human
Protein degradation
Signal transduction, biological
(distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Organelle
(endocytic vesicle, early; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Biological transport
(internalization; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Cell membrane
(lipid rafts on-; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Phosphorylation, biological
(protein; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)
IT Transforming growth factors

RL: BSU (Biological study, unclassified); BIOL (Biological study) (β-; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)

IT 74812-49-0, Ubiquitin ligase
RL: BSU (Biological study, unclassified); BIOL (Biological study) (**Smurf2**; distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)

IT 60267-61-0, Ubiquitin
RL: BSU (Biological study, unclassified); BIOL (Biological study) (distinct endocytic pathways regulate TGF-β receptor signalling and turnover as studied in mammalian cells)

RE.CNT 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Anderson, H; Mol Biol Cell 1996, V7, P1825 HCAPLUS
(2) Anderson, R; Science 1992, V255, P410 HCAPLUS
(3) Anderson, R; Science 2002, V296, P1821 HCAPLUS
(4) Baass, P; Trends Cell Biol 1995, V5, P465 HCAPLUS
(5) Benmerah, A; J Cell Biol 1998, V140, P1055 HCAPLUS
(6) Damke, H; J Cell Biol 1994, V127, P915 HCAPLUS
(7) de Renzis, S; Nature Cell Biol 2002, V4, P124 HCAPLUS
(8) Di Fiore, P; Cell 2001, V106, P1 HCAPLUS
(9) Ebisawa, T; J Biol Chem 2001, V276, P12477 HCAPLUS
(10) Ehrlich, M; J Cell Sci 2001, V114, P1777 HCAPLUS
(11) Gruenberg, J; Nature Rev Mol Cell Biol 2001, V2, P721 HCAPLUS
(12) Hayashi, H; Cell 1997, V89, P1165 HCAPLUS
(13) Hayes, S; J Cell Biol 2002, V158, P1239 HCAPLUS
(14) Henley, J; J Cell Biol 1998, V141, P85 HCAPLUS
(15) Imamura, T; Nature 1997, V389, P622 HCAPLUS
(16) Itoh, F; Genes Cells 2002, V7, P321 HCAPLUS
(17) Katzmann, D; Cell 2001, V106, P145 HCAPLUS
(18) Kavsak, P; Mol Cell 2000, V6, P1365 HCAPLUS
(19) Lamaze, C; Mol Cell 2001, V7, P661 HCAPLUS
(20) Le, P; J Biol Chem 2002, V277, P3371 HCAPLUS
(21) Levkowitz, G; Genes Dev 1998, V12, P3663 HCAPLUS
(22) Lu, Z; J Biol Chem 2002, V277, P29363 HCAPLUS
(23) Massague, J; Annu Rev Biochem 1998, V67, P753 HCAPLUS
(24) McCabe, J; Mol Biol Cell 2001, V12, P3601 HCAPLUS
(25) McPherson, P; Traffic 2001, V2, P375 HCAPLUS
(26) Miura, S; Mol Cell Biol 2000, V20, P9346 HCAPLUS
(27) Miyazono, K; Adv Immunol 2000, V75, P115 HCAPLUS
(28) Nakao, A; Nature 1997, V389, P631 HCAPLUS
(29) Nichols, B; Nature Cell Biol 2002, V4, P374 HCAPLUS
(30) Panopoulou, E; J Biol Chem 2002, V4, P18046
(31) Pelkmans, L; Nature Cell Biol 2001, V3, P473 HCAPLUS
(32) Penheiter, S; Mol Cell Biol 2002, V22, P4750 HCAPLUS
(33) Plant, P; J Cell Biol 2000, V149, P1473 HCAPLUS
(34) Prevostel, C; J Cell Sci 2000, V113, P2575 HCAPLUS
(35) Raiborg, C; EMBO J 2001, V20, P5008 HCAPLUS
(36) Razani, B; J Biol Chem 2001, V276, P6727 HCAPLUS
(37) Razani, B; J Cell Sci 2000, V113, P2103 HCAPLUS
(38) Rodal, S; Mol Biol Cell 1999, V10, P961 HCAPLUS
(39) Ros-Baro, A; Proc Natl Acad Sci USA 2001, V98, P12050 HCAPLUS
(40) Roy, S; Nature Cell Biol 1999, V1, P98 HCAPLUS
(41) Rubino, M; J Biol Chem 2000, V275, P3745 HCAPLUS
(42) Sanchez, P; Mol Cell Biol 1998, V18, P3069 HCAPLUS
(43) Sharma, P; Sem in Cell Dev Biol 2002, V13, P205 HCAPLUS
(44) Simons, K; Nature Rev Mol Cell Biol 2000, V1, P31 HCAPLUS
(45) Smart, E; Mol Cell Biol 1999, V19, P7289 HCAPLUS
(46) Subtil, A; Proc Natl Acad Sci USA 1999, V96, P6775 HCAPLUS
(47) Torgersen, M; J Cell Sci 2001, V114, P3737 HCAPLUS
(48) Tsukazaki, T; Cell 1998, V95, P779 HCAPLUS
(49) Wrana, J; Nature 1994, V370, P341 HCAPLUS
(50) Zwaagstra, J; J Biol Chem 2001, V276, P27237 HCAPLUS

L37 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2001:453687 HCAPLUS
DN 135:162954
ED Entered STN: 24 Jun 2001
TI TGF-β induces assembly of a Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation
AU Bonni, Shirin; Wang, Hong-Rui; Causing, Carrie G.; Kavsak, Peter; Stroschein, Shannon L.; Luo, Kunxin; **Wrana, Jeffrey L.**
CS Program in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5, Can.
SO Nature Cell Biology (2001), 3(6), 587-595
CODEN: NCBIFN; ISSN: 1465-7392
PB Nature Publishing Group
DT Journal
LA English
CC 2-10 (Mammalian Hormones)
AB The receptor-regulated Smad proteins are essential intracellular mediators of signal transduction by the transforming growth factor-β (TGF-β) superfamily of growth factors and are also important as regulators of gene transcription. Here we describe a new role for TGF-β-regulated Smad2 and Smad3 as components of a ubiquitin ligase complex. We show that in the presence of TGF-β signaling, Smad2 interacts through its proline-rich PPXY motif with the tryptophan-rich WW domains of **Smurf2**, a recently identified E3 ubiquitin ligases. TGF-β also induces the association of **Smurf2** with the transcriptional-co-repressor SnoN and we show that Smad2 can function to mediate this interaction. This allows **Smurf2** HECT domain to target SnoN for ubiquitin-mediated degradation by the proteasome. Thus, stimulation by TGF-β can induce the assembly of a Smad2-**Smurf2** ubiquitin ligase complex that functions to target substrates for degradation
ST TGF Smad2 **Smurf2** ubiquitin ligase SnoN
IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Smad-2; TGF-β induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)
IT Transforming proteins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(SnoN; TGF-β induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)
IT Signal transduction, biological
(TGF-β induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)
IT Transforming growth factors
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(β-; TGF-β induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)
IT 74812-49-0, Synthetase, ubiquitin-protein 140879-24-9, Proteasome
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(TGF-β induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)
RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Abdollah, S; J Biol Chem 1997, V272, P27678 HCAPLUS
(2) Akiyoshi, S; J Biol Chem 1999, V274, P35269 HCAPLUS
(3) Attisano, L; Curr Opin Cell Biol 2000, V12, P235 HCAPLUS
(4) Attisano, L; Cyto Growth Factor Rev 1996, V7, P327 HCAPLUS
(5) Bonifacino, J; Annu Rev Cell Biol 1998, V14, P19 HCAPLUS

(6) Derynck, R; Cell 1998, V95, P737 HCAPLUS
(7) Deshaies, R; Annu Rev Cell Dev Biol 1999, V15, P435 HCAPLUS
(8) Hart, M; Curr Biol 1999, V9, P207 HCAPLUS
(9) Hayashi, H; Cell 1997, V89, P1165 HCAPLUS
(10) Heldin, C; Nature 1997, V390, P465 HCAPLUS
(11) Hershko, A; Annu Rev Biochem 1998, V67, P425 HCAPLUS
(12) Imamura, T; Nature 1997, V389, P622 HCAPLUS
(13) Jackson, P; Trends Cell Biol 2000, V10, P429 HCAPLUS
(14) Kavsak, P; Mol Cell 2000, V6, P1365 HCAPLUS
(15) Kay, B; FASEB J 2000, V14, P231 HCAPLUS
(16) Kretzschmar, M; Genes Dev 1997, V11, P984 HCAPLUS
(17) Latres, E; Oncogene 1999, V18, P849 HCAPLUS
(18) Lin, X; J Biol Chem 2000, V275, P36818 HCAPLUS
(19) Lo, R; Nature Cell Biol 1999, V1, P472 HCAPLUS
(20) Luo, K; Genes Dev 1999, V13, P2196 HCAPLUS
(21) Macias-Silva, M; Cell 1996, V87, P1215 MEDLINE
(22) Massague, J; Annu Rev Biochem 1998, V67, P753 HCAPLUS
(23) Massague, J; Genes Dev 2000, V14, P627 HCAPLUS
(24) Miyazono, K; Cyto Growth Factor Rev 2000, V11, P15 HCAPLUS
(25) Nakao, A; Nature 1997, V389, P631 HCAPLUS
(26) Nomura, T; Genes Dev 1999, V13, P412 HCAPLUS
(27) Rotin, D; Current Topics in Microbiology and Immunology 1997, P115
(28) Souchelnytskyi, S; J Biol Chem 1997, V272, P28107 HCAPLUS
(29) Spencer, E; Genes Dev 1999, V13, P284 HCAPLUS
(30) Stroschein, S; Science 1999, V286, P771 HCAPLUS
(31) Sun, Y; Mol Cell 1999, V4, P499 HCAPLUS
(32) Sun, Y; Proc Natl Acad Sci USA 1999, V96, P12442 HCAPLUS
(33) Winston, J; Genes Dev 1999, V13, P270 HCAPLUS
(34) Wotton, D; Cell 1999, V97, P29 HCAPLUS
(35) Wrana, J; Cell 2000, V100, P189 HCAPLUS
(36) Xu, W; Proc Natl Acad Sci USA 2000, V97, P5924 HCAPLUS
(37) Yaron, A; Nature 1998, V396, P590 HCAPLUS
(38) Zhang, Y; Proc Natl Acad Sci USA 2001, V98, P974 HCAPLUS
(39) Zhu, H; Nature 1999, V400, P687 HCAPLUS

L37 ANSWER 6 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2001:59228 HCAPLUS
DN 134:233168
ED Entered STN: 25 Jan 2001
TI Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation
AU Kavsak, Peter; Rasmussen, Richele K.; Causing, Carrie G.; Bonni, Shirin; Zhu, Haitao; **Thomsen, Gerald H.**; **Wrana, Jeffrey L.**
CS Program in Molecular Biology and Cancer Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5, Can.
SO Molecular Cell (2000), 6(6), 1365-1375
CODEN: MOCEFL; ISSN: 1097-2765
PB Cell Press
DT Journal
LA English
CC 6-1 (General Biochemistry)
Section cross-reference(s): 7
AB Ubiquitin-mediated proteolysis regulates the activity of diverse receptor systems. Here, we identify **Smurf2**, a C2-WW-HECT domain ubiquitin ligase and show that **Smurf2** assocs. constitutively with Smad7. **Smurf2** is nuclear, but binding to Smad7 induces export and recruitment to the activated TGFβ receptor, where it causes degradation of receptors and Smad7 via proteasomal and lysosomal pathways. IFNγ, which stimulates expression of Smad7, induces Smad7-**Smurf2** complex formation and increases TGFβ receptor turnover, which is stabilized by blocking Smad7 or **Smurf2** expression. Furthermore, Smad7 mutants that interfere with recruitment of **Smurf2** to the receptors are compromised in their inhibitory

activity. These studies thus define Smad7 as an adaptor in an E3 ubiquitin-ligase complex that targets the TGFβ receptor for degradation

ST Smad7 **Smurf2** E3 ubiquitin ligase TGFbeta receptor degrdn; transforming growth factor beta receptor degrdn ubiquitin ligase Smad7

IT Transcription factors
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(Smad-7, adaptor protein; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

IT Molecular association
(Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

IT Cell nucleus
(**Smurf2** export; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

IT Cytoplasm
(cytosol, **Smurf2** export from cell nucleus to cytosol; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

IT Biological transport
(export, of **Smurf2** from cell nucleus; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

IT Transforming growth factor receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(β-transforming growth factor; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

IT Interferons
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(γ; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

IT 60267-61-0, Ubiquitin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

IT 74812-49-0, E3 Ubiquitin ligase
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(**Smurf2**; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGFβ receptor for degradation)

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

(1) Afrakhte, M; Biochem Biophys Res Comm 1998, V249, P505 HCAPLUS
(2) Anders, R; J Biol Chem 1998, V273, P23118 HCAPLUS
(3) Bitzer, M; Genes Dev 2000, V14, P187 HCAPLUS
(4) Bonifacino, J; Annu Rev Cell Dev Biol 1998, V14, P19 HCAPLUS
(5) Buschmann, T; Cell 2000, V101, P753 HCAPLUS
(6) Centrella, M; J Biol Chem 1996, V271, P18616 HCAPLUS
(7) Chen, H; Proc Natl Acad Sci USA 1995, V82, P7819
(8) Derynck, R; Cell 1998, V95, P737 HCAPLUS
(9) Hata, A; Genes Dev 1998, V12, P186 HCAPLUS
(10) Hayashi, H; Cell 1997, V89, P1165 HCAPLUS
(11) Hein, C; Mol Microbiol 1995, V18, P77 HCAPLUS
(12) Henis, Y; J Cell Biol 1994, V126, P139 HCAPLUS
(13) Hershko, A; Annu Rev Biochem 1998, V67, P425 HCAPLUS
(14) Hicke, L; Trends Cell Biol 1999, V9, P107 HCAPLUS
(15) Imamura, T; Nature 1997, V389, P622 HCAPLUS

(16) Itoh, S; J Biol Chem 1998, V273, P29195 HCAPLUS
(17) Joazeiro, C; Science 1999, V286, P309 HCAPLUS
(18) Koli, K; J Biol Chem 1997, V272, P6423 HCAPLUS
(19) Levkowitz, G; Mol Cell 1999, V4, P1029 HCAPLUS
(20) Lo, R; Nat Cell Biol 1999, V1, P472 HCAPLUS
(21) Macias-Silva, M; Cell 1996, V87, P1215 MEDLINE
(22) Massague, J; Genes Dev 2000, V14, P627 HCAPLUS
(23) Nakao, A; Nature 1997, V389, P631 HCAPLUS
(24) Springael, J; Mol Biol Cell 1998, V9, P1253 HCAPLUS
(25) Staub, O; EMBO J 1997, V16, P6325 HCAPLUS
(26) Stroschein, S; Science 1999, V286, P771 HCAPLUS
(27) Sun, Y; Proc Natl Acad Sci USA 1999, V96, P12442 HCAPLUS
(28) Tsukazaki, T; Cell 1998, V95, P779 HCAPLUS
(29) Ulloa, L; Nature 1999, V397, P710 HCAPLUS
(30) van Kerkhof, P; J Biol Chem 2000, V275, P1575 HCAPLUS
(31) Wells, R; J Biol Chem 1997, V272, P11444 HCAPLUS
(32) Wrana, J; Cell 2000, V100, P189 HCAPLUS
(33) Yokouchi, M; J Biol Chem 1999, V274, P31707 HCAPLUS
(34) Zhu, H; Nature 1999, V400, P687 HCAPLUS
(35) Zwaagstra, J; Exp Cell Res 1999, V252, P352 HCAPLUS

L37 ANSWER 7 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:900772 HCAPLUS
DN 134:53133
ED Entered STN: 22 Dec 2000
TI sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins
IN **Thomsen, Gerald H.**; **Wrana, Jeffrey**
PA Research Foundation of State University of New York, USA; HSC Research and Development Limited Partnership
SO PCT Int. Appl., 106 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N
CC 7-2 (Enzymes)
Section cross-reference(s): 12, 13
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000077168	A2	20001221	WO 2000-US16250	20000612 <--
	WO 2000077168	A3	20010503		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 2000056107	A5	20010102	AU 2000-56107	20000612 <--
	EP 1192174	A2	20020403	EP 2000-941398	20000612 <--
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI	US 1999-138969P	P	19990611 <--		
	WO 2000-US16250	W	20000612		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 2000077168	ICM	C12N

AB This invention provides unique members of the Hect family of

ubiquitin ligases that specifically target BMP and TGF<b/activin pathway-specific **Smads.** The novel ligases have been named **Smurf1** and **Smurf2.** A transgenic expression system is described for these two proteins. They directly interact with **Smads1** and 5 and **Smad7**, resp., and regulate the **ubiquitination**, turnover and activity of **Smads** and other proteins of these pathways. **Smurf1** interferes with biol. responses to BMP, but not activin signaling. In amphibian embryos **Smurf1** inhibits endogenous BMP signals, resulting in altered pattern formation and cell fate specification in the mesoderm and ectoderm. The present invention provides a unique regulatory link between the **ubiquitination** pathway and the control of cell fate determination by the TGF<b superfamily during embryonic development. Thus, **Smurf1** is a neg. regulator of **Smad1** signal transduction, by targeting **Smad1**, **Smurf1** blocks BMP signaling. **Screening** assays which survey **Smurf WW domain** interaction with **Smad** protein PPXY **domain** are also relayed. In mammalian cells, **Smurf2** suppresses TGF<b signaling, and in Xenopus, blocks formation of dorsal mesoderm and causes anterior truncation of the embryos. **Smurf2** forms a stable complex with **Smad7**, which induces degradation and downregulation of TGF<b/activin signaling. The human **Smurf1** gene was mapped to 7q21.1-q31.1.

ST human **Smurf1 Smurf2** cDNA sequence BMP TGFbeta signaling development; **ubiquitin** protein synthetase sequence human **Smurf1 Smurf2**

IT Probes (nucleic acid)
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(DNA probe identifying **Smurf** genes; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Nucleic acid hybridization
(DNA-DNA, DNA probe identifying **Smurf** genes; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**Smad7**; **Smurf1** and **Smurf3** ligase interactions with; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**Smads5**; **Smurf1** and **Smurf3** ligase interactions with; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**Smads**; **Smurf1** and **Smurf3** ligase interactions with; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Bone morphogenetic proteins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL

(Biological study); PROC (Process)
(**Smurf1** inhibiting BMP alterning mesoderm and ectoderm; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Mutation
(**Smurf1** or **Smurf2** mutation occurring at C710A or C716A; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Protein degradation
(**Smurf2** induces degradation of TGFbeta receptors and **Smad7**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Gene, animal
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**Smurf2**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Protein motifs
(assay for **screening Smurf WW domain** interaction with **Smad** protein PPXY **domain**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways)

IT Embryo, animal
(blastula, **Smurf1** mRNA localization to blastula; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(complexes, **Smurf2** forming stable complex with **Smad7** ; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Development, nonmammalian postembryonic
(developmental gene expression of **Smurf1**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Embryo, animal
(ectoderm, **Smurf1** inhibiting BMP alterning mesoderm and ectoderm; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Gene
(expression, developmental gene expression of **Smurf1**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Antibodies
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(identifying **Smurf** proteins; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Embryo, animal
(mesoderm, **Smurf1** inhibiting BMP alterning mesoderm and

ectoderm; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Genetic mapping
Genetic vectors
Protein sequences
Transformation, genetic
Xenopus laevis
cDNA sequences
(sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Gene, animal
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**smurf1**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Transforming growth factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(β-; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT 74812-49-0, Synthetase, **ubiquitin**-protein
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**Smurf1** and **Smurf2** as; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT **314013-12-2 314013-13-3**
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(amino acid sequence; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT **312903-81-4 314013-11-1**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT 60267-61-0, **Ubiquitin**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT 314014-74-9, 5: PN: WO0077168 PAGE: 47 unclaimed DNA 314014-75-0, 6: PN: WO0077168 PAGE: 48 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

L37 ANSWER 8 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:260508 HCAPLUS
DN 132:290750

ED Entered STN: 21 Apr 2000
TI Ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis
IN Zhou, Pengbo; Howley, Peter
PA President and Fellows of Harvard College, USA
SO PCT Int. Appl., 185 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N015-00
ICS C12N015-62; C12N015-12; C12N015-37; C12N015-52; C12N005-10; C07K014-00
CC 9-2 (Biochemical Methods)
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000022110	A2	20000420	WO 1999-US23705	19991008 <--
WO 2000022110	A3	20001116		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 1998-103787P P 19981009 <--
CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 2000022110	ICM	C12N015-00
	ICS	C12N015-62; C12N015-12; C12N015-37; C12N015-52; C12N005-10; C07K014-00
WO 2000022110	ECLA	C07K014/025; C07K014/47; C07K014/47A24; C12N009/00L; C12N015/62 <--

AB The present invention relates to methods and reagents for targeting proteolysis of a polypeptide by cis or trans association with a ubiquitin protein ligase. Methods and reagents for inhibiting the ubiquitination and proteolysis of cellular proteins which are recognized by a ubiquitin protein ligase are also disclosed. Thus, a gene encoding βTrCP fused to papillomavirus E7 oncoprotein N-terminus and a gene encoding the Rb protein were expressed in human osteosarcoma Saos-2 cells lacking the Rb protein. Although the Rb protein is normally stable in this cell, the protein was rapidly degraded in the transformant.
ST E3 ubiquitin ligase fusion targeted proteolysis
IT Peptidomimetics
(E3 ubiquitin ligase antagonizing; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
IT Peptides, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(E3 ubiquitin ligase antagonizing; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(E6-AP, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
IT Transcription factors

RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(E7, target protein-binding domain of, fusions with E3 ubiquitin ligase; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(IκB; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Rb, p107; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Rb; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(cullin, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Chimeric gene
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(for E3 ubiquitin ligase-targeting domain fusion; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene CDC4, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(gene E2, of human papillomavirus 16; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene EDD, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene FWD1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class

RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene HOS, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene RSP5, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene **Smurf1**, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene TOM1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene UBR1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(gene cln2; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene grr1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene met30, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene nedd-4, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL

(Biological study); PREP (Preparation)
(gene pop1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(gene pop2, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(gene sic1; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Antigens
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(large T, target protein-binding domain of, fusions with E3 ubiquitin ligase; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Protein degradation
(targeted; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, general, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Catenins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(β-; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(βTrCP, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT 143550-97-4, Protein (Saccharomyces cerevisiae clone pBM1720 gene GGR1 reduced) 169539-01-9 207624-75-7 211629-03-7 235430-49-6 264185-29-7
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(amino acid sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT 111116-07-5, DNA (Saccharomyces cerevisiae gene CDC4) 140735-75-7, GenBank M59247 202318-98-7, GenBank AF038867 206092-47-9, GenBank Y14153 225436-29-3, GenBank AF081887 264185-28-6
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT 140879-24-9, Proteasome
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(targeting proteins for degradation by; ubiquitin protein ligase-target-binding protein fusion and method for targeted

proteolysis)
IT 74812-49-0DP, E3 Ubiquitin ligase, fusions with target protein-binding domains
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
IT 255361-87-6, 1: PN: WO0001720 PAGE: 41 unclaimed DNA 255361-88-7, 5: PN: WO0001720 PAGE: 41 unclaimed DNA 255701-25-8, 9: PN: WO0001720 PAGE: 42 unclaimed DNA 264594-21-0, 7: PN: WO0022110 PAGE: 141 unclaimed DNA 264594-22-1, 8: PN: WO0022110 PAGE: 142 unclaimed DNA 264594-23-2 264594-24-3 264594-25-4 264594-26-5 264594-27-6 264594-28-7 264594-29-8 264594-30-1 264594-31-2 264594-32-3 264594-33-4 264594-34-5 264594-35-6 264863-83-4, 9: PN: WO0022110 PAGE: 142 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
IT 124585-34-8, 1-72-RNA formation factor (human immunodeficiency virus 1 clone pCV-1/pPL-12 gene tat reduced) 255385-06-9 264594-20-9
RL: PRP (Properties)
(unclaimed protein sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
IT 255039-62-4
RL: PRP (Properties)
(unclaimed sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

L37 ANSWER 9 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:234998 HCAPLUS
DN 132:329985
ED Entered STN: 12 Apr 2000
TI The Smad pathway
AU **Wrana, Jeffrey L.**; Attisano, Liliana
CS Program in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5, Can.
SO Cytokine & Growth Factor Reviews (2000), 11(1/2), 5-13
CODEN: CGFRFB; ISSN: 1359-6101
PB Elsevier Science Ltd.
DT Journal; General Review
LA English
CC 2-0 (Mammalian Hormones)
AB A review with 54 refs. Transforming growth factor-β superfamily member signals are conveyed through cell-surface serine/threonine kinase receptors to the intracellular mediators known as Smads. Activation of Smads causes their translocation from the cytoplasm to the nucleus where they function to control gene expression. In this review the authors will focus on proteins that modulate Smad activity, including SARA, for Smad Anchor for Receptor Activation, which functions during the initiation of signaling and on components of the ubiquitin-proteasome pathway, such as **Smurf1**, which can neg. regulate Smad signaling. In addition, the authors will summarize recent findings on the role of Smads as transcriptional co-modulators.
ST review Smad protein signal transduction TGF beta
IT Signal transduction, biological
(Smad signaling pathway in relation to proteins that modulate Smad activity and components of the ubiquitin-proteasome pathway)
IT Transcription factors
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(Smad; Smad signaling pathway in relation to proteins that modulate Smad activity and components of the ubiquitin-proteasome pathway)

IT Transforming growth factors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(β-; Smad signaling pathway in relation to proteins that modulate Smad activity and components of the ubiquitin-proteasome pathway)
RE.CNT 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Akiyoshi, S; J Biol Chem 1999, V274, P35269 HCAPLUS
(2) Attisano, L; Curr Op Cell Biol 1998, V10, P188 HCAPLUS
(3) Attisano, L; Cytokine and Growth Factor Reviews 1996, V7, P327 HCAPLUS
(4) Brummel, T; Genes Dev 1999, V13, P98 HCAPLUS
(5) Burd, C; Mol Cell 1998, V2, P157 HCAPLUS
(6) Chen, X; Nature 1997, V389, P85 HCAPLUS
(7) Christian, J; BioEssays 1999, V21, P382 MEDLINE
(8) Dax, P; Development 1998, V125, P1519
(9) Derynck, R; Cell 1998, V95, P737 HCAPLUS
(10) Heldin, C; Nature 1997, V390, P465 HCAPLUS
(11) Hershko, A; Annu Rev Biochem 1998, V67, P425 HCAPLUS
(12) Hocevar, B; EMBO J 1999, V18, P1345 HCAPLUS
(13) Howell, M; Dev Biol 1999, V214, P354 HCAPLUS
(14) Hua, X; Proc Natl Acad Sci 1999, V96, P13130 HCAPLUS
(15) Hudson, J; Development 1998, V125, P1407 HCAPLUS
(16) Inoue, H; Mol Biol Cell 1998, V9, P2145 HCAPLUS
(17) Kawabata, M; Cyto Growth Factor Rev 1998, V9, P49 HCAPLUS
(18) Kawabata, M; EMBO J 1998, V17, P4056 HCAPLUS
(19) Kretzschmar, M; Genes Dev 1999, V13, P804 HCAPLUS
(20) Kretzschmar, M; Nature 1997, V389, P618 HCAPLUS
(21) Krishna, S; Development 1999, V126, P251 HCAPLUS
(22) Labbe, E; Mol Cell 1998, V2, P109 HCAPLUS
(23) LeSueur, J; Development 1999, V126, P137 HCAPLUS
(24) Liu, F; Genes Dev 1997, V11, P3157 HCAPLUS
(25) Lo, R; EMBO J 1998, V17, P996 HCAPLUS
(26) Lo, R; Nature Cell Biol 1999, V1, P472 HCAPLUS
(27) Luo, K; Genes Dev 1999, V13, P2196 HCAPLUS
(28) Massague, J; Ann Rev Cell Biol 1990, V6, P597 HCAPLUS
(29) Massague, J; Annu Rev Biochem 1998, V67, P753 HCAPLUS
(30) Newfeld, S; Development 1997, V124, P3167 HCAPLUS
(31) Padgett, R; BioEssays 1998, V20, P382 MEDLINE
(32) Padgett, R; Cyto Growth Factor Rev 1997, V8, P1 HCAPLUS
(33) Roberts, A; Growth Factors 1993, V8, P1 MEDLINE
(34) Sano, Y; J Biol Chem 1999, V274, P8949 HCAPLUS
(35) Sekelsky, J; Genetics 1995, V139, P1347 HCAPLUS
(36) Shi, Y; Cell 1998, V94, P585 HCAPLUS
(37) Shi, Y; Nature 1997, V388, P87 HCAPLUS
(38) Stroschein, S; Science 1999, V286, P771 HCAPLUS
(39) Sun, Y; Mol Cell 1999, V4, P499 HCAPLUS
(40) Sun, Y; Proc Natl Acad Sci 1999, V96, P12442 HCAPLUS
(41) ten Dijke, P; Nature 1999, V397, P109 HCAPLUS
(42) Tsukazaki, T; Cell 1998, V95, P779 HCAPLUS
(43) Tsuneizumi, K; Nature V389, P627 HCAPLUS
(44) Waltzer, L; EMBO J 1999, V18, P1630 HCAPLUS
(45) Whitman, M; Genes and Dev 1998, V12, P2445 HCAPLUS
(46) Wiedemann, C; Nature 1998, V394, P426 HCAPLUS
(47) Wisotzkey, R; Development 1998, V125, P1433 HCAPLUS
(48) Wotton, D; Cell 1999, V97, P29 HCAPLUS
(49) Wu, G; Science 2000, V287, P92 HCAPLUS
(50) Yanagisawa, J; Science 1999, V283, P1317 HCAPLUS
(51) Yeo, C; J Biol Chem 1999, V274, P26584 HCAPLUS
(52) Zhang, Y; Nature 1998, V394, P909 HCAPLUS
(53) Zhou, S; Mol Cell 1998, V2, P121 HCAPLUS
(54) Zhu, H; Nature 1999, V400, P687 HCAPLUS

L37 ANSWER 10 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN

AN 1999:544811 HCAPLUS
DN 131:284125
ED Entered STN: 30 Aug 1999
TI A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation
AU Zhu, Haitao; Kavsak, Peter; Abdollah, Shirin; **Wrana, Jeffrey L.**; **Thomsen, Gerald H.**
CS Department of Biochemistry and Cell Biology and Institute for Cell and Developmental Biology, State University of New York, Stony Brook, NY, 11794-5215, USA
SO Nature (London) (**1999**), 400(6745), 687-693
CODEN: NATUAS; ISSN: 0028-0836
PB Macmillan Magazines
DT Journal
LA English
CC 12-3 (Nonmammalian Biochemistry)
Section cross-reference(s): 3, 7
AB TGF-β-like factors signal across cell membranes through complexes of transmembrane receptors known as type I and type II serine/threonine-kinase receptors, which in turn activate the SMAD signaling pathway. On the inside of the cell membrane, a receptor-regulated class of SMADs are phosphorylated by the type-I-receptor kinase. In this way, receptors for different factors are able to pass on specific signals along the pathway: for example, receptors for bone morphogenetic protein (BMP) target SMADs 1, 5, and 8, whereas receptors for activin and TGF-β target SMADs 2 and 3. Phosphorylation of receptor-regulated SMADs induces their association with Smad4, the common-partner SMAD, and stimulates accumulation of this complex in the nucleus, where it regulates transcriptional responses. Here we describe **Smurf1**, a new member of the Hect family of E3 ubiquitin ligases. **Smurf1** selectively interacts with receptor-regulated SMADs specific for the BMP pathway to trigger their ubiquitination and degradation, and hence their inactivation. In the amphibian Xenopus laevis, **Smurf1** mRNA is localized to the animal pole of the egg; in Xenopus embryos, ectopic **Smurf1** inhibits the transmission of BMP signals and thereby affects pattern formation. **Smurf1** also enhances cellular responsiveness to the Smad2 (activin/TGF-β) pathway. Thus, targeted ubiquitination of SMADs may serve to control both embryonic development and a wide variety of cellular responses to TGF-β signals.
ST frog embryo signaling ubiquitin ligase; sequence E3 ubiquitin ligase Xenopus
IT Cell nucleus
Development, nonmammalian postembryonic
Egg
Eye
Kidney
Molecular cloning
Protein sequences
Signal transduction, biological
Transcriptional regulation
Xenopus laevis
cDNA sequences
(SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
IT Bone morphogenetic proteins
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
IT Bone morphogenetic protein receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(SMAD ubiquitin ligase sequence and targeting of BMP pathway and role

in embryonic pattern formation in frogs)

IT Transcription factors
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Smad-1; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Transcription factors
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Smad-2; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Transcription factors
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Smad-5; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Gene, animal
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(**Smurf1**; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Embryo, animal
(branchial arch; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Nervous system
(central; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Embryo, animal
(ectoderm; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Embryo, animal
(embryogenesis; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Gene
(expression; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Embryo, animal
(mesoderm; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Phosphorylation, biological
(protein; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT Embryo, animal
(somite; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT 74812-49-0, E3 Ubiquitin ligase
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT **246223-04-1**
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(amino acid sequence; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT **237728-87-9**, GenBank AF169310
RL: PRP (Properties)
(nucleotide sequence; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
RE.CNT 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Bartel, P; Methods Enzymol 1995, V254, P241 HCAPLUS
(2) Candia, A; Development 1997, V124, P4467 HCAPLUS
(3) Eppert, K; Cell 1996, V86, P543 HCAPLUS
(4) Fainsod, A; EMBO J 1994, V13, P5015 HCAPLUS
(5) Fenteany, G; Science 1995, V268, P726 HCAPLUS
(6) Harland, R; Annu Rev Cell Biol 1997, V13, P611 HCAPLUS
(7) Hein, C; Mol Microbiol 1995, V18, P77 HCAPLUS
(8) Hemmati-Brivanlou, A; Cell 1997, V88, P13 HCAPLUS
(9) Hemmati-Brivanlou, A; Dev Genet 1995, V17, P78 HCAPLUS
(10) Hershko, A; Annu Rev Biochem 1998, V67, P425 HCAPLUS
(11) Hoodless, P; Dev Biol 1999, V207, P364 HCAPLUS
(12) Huibrcgtse, J; Proc Natl Acad Sci 1995, V92, P2563
(13) Huibrcgtse, J; Proc Natl Acad Sci 1995, V92, P5249
(14) Kingsley, D; Genes Dev 1994, V8, P133 HCAPLUS
(15) Lagna, G; Nature 1996, V383, P832 HCAPLUS
(16) Macias-Silva, M; J Biol Chem 1998, V273, P25628 HCAPLUS
(17) Massague, J; Annu Rev Biochem 1998, V67, P753 HCAPLUS
(18) Moses, H; Curr Opin Genet Dev 1996, V6, P581 HCAPLUS
(19) Nalefski, E; Protein Sci 1996, V5, P2375 HCAPLUS
(20) Nefsky, B; EMBO J 1996, V15, P1301 HCAPLUS
(21) Nieuwkoop, P; Normal Table of Xenopus laevis 1967
(22) Nishimatsu, S; Mech Dev 1997, V74, P75
(23) Plant, P; J Biol Chem 1997, V272, P32329 HCAPLUS
(24) Rotin, D; Curr Top Microbiol Immunol 1998, V228, P115 HCAPLUS
(25) Scheffner, M; Cell 1993, V75, P495 HCAPLUS
(26) Suzuki, A; Dev Biol 1997, V184, P402 HCAPLUS
(27) Thomsen, G; Development 1996, V122, P2359 HCAPLUS
(28) Treier, M; Cell 1994, V78, P787 HCAPLUS
(29) Ward, C; Cell 1995, V83, P121 HCAPLUS
(30) Whitman, M; Genes Dev 1998, V12, P2445 HCAPLUS
(31) Wilson, P; Development 1997, V124, P3177 HCAPLUS

L37 ANSWER 11 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1997:335178 HCAPLUS
DN 126:303135
ED Entered STN: 29 May 1997
TI Eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses
IN Beach, David; Caligiuri, Maureen; Nefsky, Bradley
PA Cold Spring Harbor Laboratory, USA; Beach, David; Caligiuri, Maureen; Nefsky, Bradley
SO PCT Int. Appl., 109 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N009-00
ICS C12Q001-25
CC 7-2 (Enzymes)
Section cross-reference(s): 3, 10, 13
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9712962	A1	19970410	WO 1996-US15930	19961004 <--
	W: CA, JP, MX, US				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 6001619	A	19991214	US 1995-539205	19951004 <--

	CA 2231645	AA	19970410	CA 1996-2231645	19961004 <--
	EP 857205	A1	19980812	EP 1996-933244	19961004 <--
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	US 6503742	B1	20030107	US 1999-392163	19990908 <--
	US 2003199036	A1	20031023	US 2002-313955	20021205 <--
PRAI	US 1995-539205	A	19951004 <--		
	WO 1996-US15930	W	19961004 <--		
	US 1999-392163	A1	19990908		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 9712962	ICM	C12N009-00
	ICS	C12Q001-25
US 6001619	ECLA	C12N009/00L; G01N033/50D2 <--
US 6503742	ECLA	C12N009/00L; C12Q001/25; G01N033/68 <--
US 2003199036	ECLA	C12N009/00L; C12Q001/25; G01N033/68 <--

AB The present invention relates to the discovery in eukaryotic cells of ubiquitin ligases. These proteins are referred to herein collectively as "pub" proteins for Protein UBiquitin ligase, and individually as h-pub1, h-pub2, h-pub3 and s-pub1 for the human pub1, pub2 an pub3 and Schizosaccharomyces pombe pub1 clones, resp. Pub1 proteins apparently play a role in the ubiquitination of the mitotic activating tyrosine phosphatase cdc25, and thus they may regulate the progression of proliferation in eukaryotic cells by activating the cyclin dependent kinase complexes. In S. pombe, disruption of s-pub1 elevates the level of cdc25 protein in vivo increasing the activity of the tyrosine kinases. Wee1 and mik1, required to arrest the cell-cycle. Loss of wee1 function in an S. pombe cell carrying a disruption in the s-pub1 gene results in a lethal premature entry into mitosis; such lethal phenotype can be rescued by the loss of cdc25 function. A ubiquitin thioester adduct of s-pub1 can be isolated from S. pombe and disruption of s-pub1 dramatically reduces ubiquitination of cdc25.

ST ubiquitin ligase gene pub Schizosaccharomyces human; cDNA sequence ubiquitin ligase Schizosaccharomyces human; tyrosine phosphatase cdc25 ubiquitin ligase pub; kinase tyrosine wee1 mik1 ubiquitin ligase; mitosis ubiquitin ligase pub Schizosaccharomyces human

IT Genetic mapping
(Schizosaccharomyces pombe gene pub1 mapping on chromosome 1 right arm; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Cell proliferation
Eukaryote (Eukaryotae)
Mitosis
Protein sequences
Schizosaccharomyces pombe
cDNA sequences
(eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Genetic vectors
(expression; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Proteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(gene wee1, activation by cdc25; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Chromosome
(microbial, Schizosaccharomyces pombe gene pub1 mapping on chromosome 1

right arm; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Cell cycle
(progression; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Gene, animal
Gene, microbial
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)
(pub1; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Gene, animal
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)
(pub2; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Gene, animal
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)
(pub3; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Animal
(transgenic, expression host; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Enzymes, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(ubiquitin-activating, ubiquitin conjugation system use; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Enzymes, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(ubiquitin-conjugating, ubiquitin conjugation system use; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT p53 (protein)
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(ubiquitination; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Fusion proteins (chimeric proteins)
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(with ubiquitin ligase; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT 144114-10-3, Protein kinase mik1

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(activation by cdc25; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT **189284-44-4P** 189284-45-5P 189284-46-6P 189284-47-7P
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)
(amino acid sequence; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT 74812-49-0P, Ubiquitin-protein ligase
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)
(eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT 60267-61-0D, Ubiquitin, gene pub1 ubiquitin ligase thioester adducts
74812-49-0D, Ubiquitin-protein ligase, ubiquitin thioester adducts
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); OCCU (Occurrence)
(eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT 189284-40-0 189284-41-1 189284-42-2 189284-43-3
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)
(nucleotide sequence; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT 9000-83-3, ATPase 60267-61-0, Ubiquitin
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(ubiquitin conjugation system use; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT 140208-22-6, Gene cdc25 phosphatase
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(ubiquitination; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

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AN 1999:431362 BIOSIS
DN PREV199900431362
TI A **SMAD** ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation.
AU Zhu, Haitao; Kavsak, Peter; Abdollah, Shirin; **Wrana, Jeffrey L.**; **Thomsen, Gerald H.** [Reprint author]
CS Department of Biochemistry and Cell Biology and Institute for Cell and Developmental Biology, State University of New York, Stony Brook, NY, 11794-5215, USA
SO Nature (London), (Aug. 12, 1999) Vol. 400, No. 6745, pp. 687-693. print.
CODEN: NATUAS. ISSN: 0028-0836.
DT Article
LA English
ED Entered STN: 18 Oct 1999
Last Updated on STN: 18 Oct 1999
AB The TGF-beta superfamily of proteins regulates many different biological processes, including cell growth, differentiation and embryonic pattern formation. TGF-beta-like factors signal across cell membranes through complexes of transmembrane receptors known as type I and type II serine/threonine-kinase receptors, which in turn activate the **SMAD** signalling pathway. On the inside of the cell membrane, a receptor-regulated class of **SMADs** are phosphorylated by the type-I-receptor kinase. In this way, receptors for different factors are able to pass on specific signals along the pathway: for example, receptors for bone morphogenetic protein (BMP) target **SMADs** 1, 5 and 8, whereas receptors for activin and TGF-beta target **SMADs** 2 and 3. Phosphorylation of receptor-regulated **SMADs** induces their association with **Smad4**, the 'common-partner' **SMAD**, and stimulates accumulation of this complex in the nucleus, where it regulates transcriptional responses. Here we describe **Smurf1**, a new member of the Hect family of E3 ubiquitin ligases. **Smurf1** selectively interacts with receptor-regulated **SMADs** specific for the BMP pathway in order to trigger their ubiquitination and degradation, and hence their inactivation. In the amphibian Xenopus laevis, **Smurf1** messenger RNA is localized to the animal pole of the egg; in Xenopus embryos, ectopic **Smurf1** inhibits the transmission of BMP signals and thereby affects pattern formation. **Smurf1** also enhances cellular responsiveness to the **Smad2** (activin/TGF-beta) pathway. Thus, targeted ubiquitination of **SMADs** may serve to control both embryonic development and a wide variety of cellular responses to TGF-beta signals.
CC Cytology - Animal 02506
Biochemistry methods - General 10050
Biochemistry studies - General 10060
Biophysics - General 10502
Enzymes - General and comparative studies: coenzymes 10802
Development and Embryology - General and descriptive 25502
General biology - Miscellaneous 00532
IT Major Concepts
Cell Biology; Development; Enzymology (Biochemistry and Molecular Biophysics)
IT Chemicals & Biochemicals
bone morphogenetic protein [BMP]; **SMAD** ubiquitin ligase: BMP pathway targeting
IT Miscellaneous Descriptors
embryonic pattern formation
ORGN Classifier

```
        Cercopithecidae   86205
     Super Taxa
        Primates; Mammalia; Vertebrata; Chordata; Animalia
     Organism Name
        COS-1 cell line: African green monkey cells
     Taxa Notes
        Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates,
        Nonhuman Primates, Primates, Vertebrates
ORGN Classifier
        Mammalia   85700
     Super Taxa
        Vertebrata; Chordata; Animalia
     Organism Name
        293T cell line: mammalian cells
     Taxa Notes
        Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals,
        Vertebrates
ORGN Classifier
        Salientia   85306
     Super Taxa
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        Xenopus
     Taxa Notes
        Amphibians, Animals, Chordates, Nonhuman Vertebrates, Vertebrates


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L46  ANSWER 1 OF 7  WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN   2004-625495 [60]   WPIX
DNC  C2004-225008
TI   Decreasing infection of cell by virus, HIV, influenza A or Ebola,
     comprises interfering with activity or expression of host proteins or
     activity of host nucleic acids such as Rab9, AXL receptor tyrosine kinase,
     and Beta-chimerin .
```

DC B04 D16
IN HODGE, T W; MOREY, N J; RUBIN, D; SANCHEZ, A; SHAW, M W
PA (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC 107
PI WO 2004070002 A2 20040819 (200460)* EN 396 C12N000-00
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE
LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM
PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US
UZ VC VN YU ZA ZM ZW
ADT WO 2004070002 A2 WO 2003-US37143 20031118
PRAI US 2003-482604P 20030625; US 2002-427464P 20021118
IC ICM C12N000-00
AB WO2004070002 A UPAB: 20040920
NOVELTY - Decreasing infection of a host cell by a virus comprises interfering with an activity or expression of one or more host proteins or interfering with an activity of one or more host nucleic acids where the host protein or nucleic acid comprises Rab9, AXL receptor tyrosine kinase, Beta-chimerin and mammalian selenium binding protein.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) methods of decreasing HIV, Ebola, or influenza A infection of a host cell;

(2) a method of treating an HIV, Ebola, or influenza A viral infection in a host subject;

(3) a method of determining resistance or susceptibility to viral infection in a subject;

(4) a method of identifying a compound that decreases binding of a viral protein to a host protein and decreases viral infection;

(5) a method of decreasing infection of a host cell by a pathogen;

(6) a cell comprising a functional deletion of one or more target sequences associated with any of the 35 nucleotide sequences fully defined in the specification, where the cell has a decreased susceptibility to HIV infection;

(7) a cell comprising a functional deletion of one or more target sequences associated with any of the 27 nucleotide sequences fully defined in the specification, where the cell has a decreased susceptibility to influenza infection;

(8) a cell comprising a functional deletion of one or more target sequences associated with any of the 168 nucleotide sequences fully defined in the specification, where the cell has a decreased susceptibility to Ebola infection;

(9) a cell comprising a functional deletion of a Rab9 gene, where the cell has a decreased susceptibility to infection by a pathogen that uses lipid rafts; and

(10) a non-human transgenic mammal comprising any of the functional deletions cited above.

ACTIVITY - Virucide; Anti-HIV; Antibacterial.

MECHANISM OF ACTION - RNAi; RNA interference; Axl tryosine kinase receptor inhibitor; Rab9 inhibitor; beta chimerin inhibitor; retinoblastoma binding protein 1 inhibitor; protein cell control modulator; mammalian selenium binding protein inhibitor; KOX inhibitor.

Rab9, AXL (AXL receptor tyrosine kinase), CHN (Beta-chimerin), KOX, RBB (retinoblastoma binding protein 1), KIAA1259, F3 and mammalian selenium binding protein siRNA sequences were generated, pooled, hybridized to its appropriate complement sequence and used to transfect JC53 (HeLa cells modified to accept HIV), Vero (monkey kidney cells), MDCK (dog kidney cells, or HEK (human kidney cells). GFP siRNA sequences were used as negative controls.

Cells (20000 to 250000) were incubated in serum free media for 24 hours. Cocktails were made by mixing the siRNAs (50-100 pmoles) with

lipofectamine 2000 (4-16 micro l) and RNAse inhibitor (1-4 micro l) in a solution of Optimem (serum free medium) in a total volume of 200-2000 micro l. Aliquots (50-500 micro l) of the cocktail were added to the cells which were incubated at 37 deg. C for 48 hours. The cells were then infected with HIV, Ebola, or influenza and the incubation continued for 3-7 days. Following transfection, several assays were conducted to confirm transfection efficiency and to determine the resistance of the cells to infection by various agents.

Quantitation of p24 levels of HIV infected J5C3 cells was determined. Rab9 siRNAs and mammalian selenium binding protein siRNAs each decreased HIV infection by 50% on day 4 post infection (day 7 post addition of siRNA). In addition, HIV infection decreased by 80-90% in the presence of beta-chimerin siRNAs, KOX siRNAs, or retinoblastoma binding protein 1 siRNA. However, HIV infection did not decrease in the presence of siRNAs that recognize KIAA1259, F3 or AXL siRNAs.

Infection of Ebola in HEK293 cells transfected with Rab9 or AXL siRNA was determined by measuring gp1 antigen using fluorescent antibody to gp1 envelope protein. Infection was decreased by 90-95% in presence of Rab9 siRNA, as compared to infection in absence of Rab9. Infection decreased by 80% in presence of AXL siRNA compared to absence.

USE - The method is useful for decreasing and treating infection of a host cell by a virus, such as HIV, influenza A or Ebola virus. Specifically, especially where the pathogen hijacks a lipid raft, the method is useful for decreasing infection of Campylobacter jejuni, Vibrio cholerae SV40, Legionella pneumophila, Aeromonas hydrophila, Echovirus 1, Echovirus 11, Brucella spp., Clostridium spp., Avian sarcoma and leukosis virus, FimH, Escherichia coli, Streptococcus pyogenes, Semliki forest virus, Salmonella typhimurium, Bacillus anthracis, Ecotropic mouse leukaemia virus, Shigella flexneri, Bacillus thuringiensis, HTLV-I, Chlamydia spp., Helicobacter pylori, HFV-I, Mycobacterium spp., Listeria monocytogenes, Ebola, Marburg, Measles, Herpes Simplex virus, influenza virus, or Epstein-Barr virus (claimed).

Dwg.0/6

FS CPI

FA AB

MC CPI: B04-E03; B04-E06; B04-E07; B04-F0200E; B04-G01; B04-N02; B04-N03; B04-P01A0E; B11-C08E; B12-K04A; B12-K04E; B14-A01; B14-A02; D05-H09; D05-H11; D05-H12D2; D05-H12D6; D05-H14B2; D05-H16A

TECH UPTX: 20040920

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: In decreasing infection of a host cell by a virus, the host protein or host nucleic acid is a T-cell receptor V beta chain; T-cell receptor V-D-J beta 2.1 chain; beta-chimerin; malic enzyme 1; hypothetical protein XP174419; sequence from chromosome 4q31.3-32; alpha satellite DNA; LOC253788; LOC219938; coagulation factor m (F3); LOC91759; similar to KOX4 (LOC131880); LOC166140; LOC222474; similar to Rho guanine nucleotide exchange factor 4, isoform a; APC-stimulated guanine nucleotide exchange factor (LOC221178); T-cell receptor beta; ribosomal protein L7A-like 4; v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian) (SRC); KIAA0564; alpha satellite DNA; M96 protein; hypothetical protein similar to G proteins (LOC57826); LOC161005; osteoblast specific factor 2; Canis familiaris T-cell leukemia translocation-associated protein; aminomethyltransferase; dystroglycan; bassoon; LIM domain containing preferred translocation partner in lipoma; sequence between LOC253121 and hyaluronan synthase 2; testin 2, testin 3; protein tyrosine phosphatase, non-receptor type 1; sequence between LOC149360 and LOC253961; sequence between KIAA1560 and tectorin beta; cadherin related 23; myeloid/lymphoma or mixed lineage leukemia, translocated to 10; exportin 5; DNA polymerase eta (POLH); heterogenous nuclear riboprotein C (C1/C2); alpha-endosulfine pseudogene; LOC128741; LOC222888; LOC138421; zinc finger protein 297B; sideroflexin 5; importin 9 (FLJ10402); T-cell receptor beta; similar to murine putative transcription factor ZNF131 (LOC135952); KIAA1259; MURR1; CCT4; FLJ40773; similar to ribosomal protein L24-like (LOC149360); polybromo 1; DNA damage

inducible transcript 3; KIAA1887; PDZ ; LIM domain 1 (elfin); LOC284803; PRO0097; FLJ31958; small inducible cytokine E, member 1 (endothelial monocyte-activating); E3 ubiquitin ligase (**SMURF2**); MGC40489; Rab9; PRO1617; retinoblastoma binding protein 1; region of chromosome 2q12; elongation factor for selenoprotein translation; Transcription factor SMIF (HSA275986); KIAA1026; trinucleotide repeat containing 5 (TNRC5); homogentisate 1,2-dioxygenase (HGD); region of chromosome Xq23-24; region of chromosome 4p15.3; similar to LWamide neuropeptide precursor protein (Hydractinia echinata) (LOC129883); region of chromosome 2q21; region of chromosome XpI 1.4, including UPS9X; LOC221829 ; U3 small nuclear RNA; integral, beta 1 (ITGB1) ; acrosomal vesicle protein 1 (ACRV1) and CHK1 checkpoint homolog (CHEK1); prospero-related homeobox 1 (PROX1); FLJ20627 and FLJ12910; PIN2-interacting protein (PINX1) and SRY (sex-determining region Y)-box 7 (SOX7); LOC131920; region of chromosome 13q14; neurotrophic tyrosine kinase, receptor, type 3 (NTRK3); TERA protein and FLJ13224; LOC284260; POM (POM121 homolog) and ZP3 fusion (POMZP3); DEAD/H box polypeptide 8 (DDX8) and similar to ribosomal protein L29 (cell surface heparin binding protein HIP) (LOC284064); LOC345307 and UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GALNT7); Mus musculus 5S rRNA pseudogene (Rn5s-ps1); ribosomal protein L27a pseudogene (RPL27AP) and v-myb myeloblastosis viral oncogene homolog-like 2 (MYBL2); Down's syndrome cell adhesion molecule like 1 (DSCAML1); LOC148529; Huntingtin-associated protein interacting protein (HAPIP); LOC158525 and similar to RIKEN cDNA 1210001E11 (LOC347366); hypothetical protein FLJ12910; LOC350411; allograft inflammatory factor 1 (AIF1) and HLA-B associated transcript 2 (BAT2); C10orf7; LOC346658 and LOC340349; region of chromosome 12q21; LOC339248 and FLJ22659; SR rich protein DKFZp564B0769 and hypothetical protein MGC14793; FLJ10439; cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP1 IA1) and sema domain, immunoglobulin domain (Ig) and GPI membrane anchor, (semaphoring) 7A; ribosomal protein S16 (RPS 16); hypothetical protein DKFZp434H0115 and ATP citrate lyase (ACLY); calnexin (CANX); protein tyrosine phosphatase, receptor type, K (PTPRK); cyclin M2 (CNNM2); or AXL receptor tyrosine kinase (AXL), and where interfering with the activity or expression of the one or more host proteins decreases infection of the host cell by the virus. The one or more host proteins is encoded by one or more host nucleic acids comprising, or having at least at least 90% identity to any target nucleic acid sequence associated with any of the 229 sequences (S1-S229), fully defined in the specification. The method comprises interfering with an activity or expression of more than one, or at least three of the host proteins. The virus is HIV-I or HIV-2. The method comprises interfering with expression of one or more of the host nucleic acids. The virus is influenza A, and the host protein is a Canis familiaris T-cell leukemia translocation-associated protein, aminomethyltransferase; dystroglycan; bassoon; LJM domain containing preferred translocation partner in lipoma; sequence between LOC253121 and hyaluronan synthase 2; testin 2; testin 3; PTPN1 gene for protein tyrosine phosphatase, non-receptor type 1; sequence between LOC1493 60 and LOC253961; sequence between KIAA1560 and tectorin beta; cadherin related 23; malic enzyme 1; hypothetical protein XP174419; sequence from chromosome 4q31.3-32; Rab9, or a myeloid/lymphoma or mixed lineage leukemia, translocated to 10. The virus is Ebola, and the host protein is a exportin 5; DNA polymerase eta (POLH); heterogenous nuclear riboprotein C; alpha-endosulfine pseudogene; LOC128741; LOC222888; LOC138421; zinc finger protein 2977B; sideroflexin 5; importin; (FLJ10402); T-cell receptor beta; similar to murine putative transcription factor ZNF131 (LOC135952); KIAA1259; MURR1; CCT4; FLJ40773; ribosomal protein L24-like (LOC149360); testin 2; testin 3; polybromo 1; DNA damage inducible transcript 3; KIAA1887; PDZ; LIM domain 1 (elfin); LOC284803; PRO0097; FLJ31958; small inducible cytokine E, member 1 (endothelial monocyte-activating); E3 ubiquitin ligase; MGC40489; Rab9; PRO1617; retinoblastoma binding protein 1; region of chromosome 2q12; elongation factor for selenoprotein translation; Transcription factor SMIF

(HSA275986); KIAA1026; trinucleotide repeat containing 5 (TNRC5); homogentisate 1,2-dioxygenase (HGD); region of chromosome Xq23-24; region of chromosome 4pl5.3; similar to LWamide neuropeptide precursor protein (Hydractinia echinata) (LOC129883); region of chromosome 2q21; region of chromosome Xp 11.4, including UPS9X; L(tm)C221829 ; U3 small nuclear RNA; integral, beta 1 (ITGBl); acrosomal vesicle protein 1 (ACRV1) and CHKl checkpoint homolog (CHEKl); prospero-related homeobox 1 (PROXl); FLJ20627 andFLJ12910; PIN2-interacting protein (PINXl) and SRY (sex-determining region Y)-box 7 (SOX7); LOC131920; region of chromosome 13ql4; neurotrophic tyrosine kinase, receptor, type 3 (NTRK3); TERA protein and FLJ13224; LOC284260; POM (POM121 homolog) and ZP3 fusion (POMZP3); DEAD/H box polypeptide 8 (DDX8) and similar to ribosomal protein L29 (cell surface heparin binding protein HIP) (LOC284064); LOC345307 and UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GALNT7); Mus musculus 5S rRNA pseudogene (Rn5s-psl); ribosomal protein L27a pseudogene (RPL27AP) and v-myb myeloblastosis viral oncogene homolog-like 2 (MYBL2); Down's syndrome cell adhesion molecule like 1 (DSCAML1); LOC148529; Huntingtin-associated protein interacting protein (HAPIP); LOCI 58525 and similar to RIKEN cDNA 1210001E11 (LOC347366); hypothetical protein FLJ12910; LOC350411; allograft inflammatory factor 1 (AIFl) and HLA-B associated transcript 2 (BAT2); ClOorfJ; LOC346658 and LOC340349; region of chromosome 12q21; LOC339248 andFLJ22659; SR rich protein DKFZp564B0769 and hypothetical protein MGC14793; FLJ10439; cytochrome P450, family 11, subfamily A, polypeptide (CYPl IAl) and sema domain, immunoglobulin domain (Ig) and GPI membrane anchor, (semaphoring) 7A; ribosomal protein S16 (RPS16); hypothetical protein DKFZp434H0115 and ATP citrate lyase (ACLY); calnexin (CANX); protein tyrosine phosphatase, receptor type, K (PTPRK); cyclin M2 (CNNM2); or AXL receptor tyrosine kinase.

Interfering with the activity of the one or more host proteins comprises decreasing an interaction of a viral protein and the one or more host proteins by disrupting or decreasing expression of the one or more host proteins. The viral protein comprises a virus and decreasing the interaction of the viral protein and the one or more host proteins decreases or inhibits infection of a host cell by the virus. Disrupting or decreasing expression of the host protein comprises disrupting or decreasing transcription of an mRNA encoding the host protein. Disrupting or decreasing transcription of the mRNA comprises inserting a transposon or insertional vector into a coding region of the nucleic acid encoding the host protein. Disrupting or decreasing the transcription of the mRNA comprises contacting the mRNA with an antisense RNA, RNAi, ribozyme, or siRNA that recognizes the mRNA. Interfering with the activity of the host protein comprises decreasing an interaction of a viral protein and the host protein by contacting the cell with an agent that decreases or inhibits the activity or expression of the host protein or that disrupts expression of the host protein. The host cell is present in a host subject and where contacting the cell with the agent comprises administering the agent to the subject. The host cell is a mammalian host cell.

Decreasing HIV, Ebola, or influenza A infection of a host cell comprises decreasing an interaction between a viral nucleic acid and a host nucleic acid by decreasing the integration of the viral nucleic acid into the host nucleic acid. The viral nucleic acid comprises a viral genome and the host nucleic acid comprises a host genome. This method alternatively comprises contacting the host cell with an anti-protein binding agent that selectively or specifically binds to a host protein encoded by any target sequence associated with S1-S229, where the anti-protein binding agent inhibits an interaction between the host protein and the HIV, Ebola, or influenza A virus. The host cell is present in a subject, and contacting the host cell with the anti-protein binding agent comprises administering the anti-protein binding agent to the subject. The anti-protein binding agent is an antibody or chemical compound.

Treating an HIV, Ebola, or influenza A viral infection in a host subject comprises administering to a subject having a viral infection an effective

amount of an agent that interferes with the interaction of a virus and host protein. The agent disrupts expression of the nucleic acid encoding the host protein. The agent is an antisense, ribozyme, or siRNA molecule that recognizes the nucleic acid sequence comprising at least 90% identity to any target sequence associated with S1-S229. The effective amount induces a prophylactic effect in the host, which inhibits infection of the host by a virus. The host was previously infected by a virus and the effective amount induces a therapeutic effect in the host.
Determining resistance or susceptibility to viral infection in a subject comprises comparing a first nucleic acid sequence of a subject to a second nucleic acid sequence comprising any target sequence associated with S1-S229, where a higher similarity between the first and second nucleic acid sequence indicates the subject is more susceptible to viral infection, and where a lesser similarity between the first and second nucleic acid sequence indicates the subject is more resistant to viral infection. The first nucleic acid sequence is obtained from a biological sample of the subject. The first nucleic acid sequence comprises a plurality of nucleic acid sequences, where each nucleic acid sequence is obtained from a different subject. This method further comprises determining a polymorphic variation within a population.
Identifying a compound that decreases binding of a viral protein to a host protein and decreases viral infection comprises contacting the host protein with the viral protein and a test compound, wherein the host protein is any of the protein listed in the specification, and the viral protein is an HIV, Ebola, or influenza A protein; and determining whether binding of the viral protein to the host protein is decreased in the presence of the test compound, the decrease in binding being an indication that the test compound decreases the binding of viral protein to the target protein, and decreases viral infection. The viral protein comprises a virus. The viral protein is a viral envelope protein. The viral protein is an HIV protein. This method comprises expressing the host protein in a cell, and contacting the host protein with the viral protein and a test compound comprises exposing the cell to the viral protein and the test compound. The host protein or the viral protein comprises a label, and determining whether binding is decreased comprises detecting an amount of label present.
Decreasing infection of a host cell by a pathogen comprises interfering with an activity or expression of a Rab9 in the host cell, where interfering with Rab9 activity or expression decreases infection of the host cell by the pathogen. The pathogen hijacks a lipid raft. The pathogen is a Campylobacter jejuni, Vibrio cholerae SV40, Legionella pneumophila, Aeromonas hydrophila, Echovirus 1, Echovirus 11, Brucella spp., Clostridium spp., Avian sarcoma and leukosis virus, FimH, Escherichia coli, Streptococcus pyogenes, Semliki forest virus, Salmonella typhimurium, Bacillus anthracis, Ecotropic mouse leukemia virus, Shigella flexneri, Bacillus thuringiensis, HTLV-I, Chlamydia spp., Helicobacter pylori, HFV-I, Mycobacterium spp., Listeria monocytogenes, Ebola, Marburg, Measles, Herpes Simplex virus, influenza virus, or Epstein-Barr virus. Interfering with expression of Rab9 comprises disrupting or decreasing transcription of an mRNA encoding the Rab9 protein. Disrupting or decreasing the transcription of the mRNA comprises contacting the mRNA with an antisense RNA, ribozyme, or siRNA that recognizes the mRNA. The host cell is present in a subject, and contacting the mRNA with an antisense RNA, ribozyme, or siRNA that recognizes the mRNA comprises administering the antisense RNA, ribozyme, or siRNA to the subject.

L46 ANSWER 2 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2004-315601 [29] WPIX
DNN N2004-251489 DNC C2004-119632
TI Identifying protein-protein interactions, useful e.g. in drug development, comprises introducing into cells one or more prey proteins labeled with an epitope tag and one or more bait proteins labeled with a detectable substance.

DC B04 D16 S03
IN BARRIOS-RODILES, M; WRANA, J
PA (MOUN) MOUNT SINAI HOSPITAL
CYC 105
PI WO 2004023146 A2 20040318 (200429)* EN 53 G01N033-68
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS
LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH
PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC
VN YU ZA ZM ZW
AU 2003264211 A1 20040329 (200459) G01N033-68
ADT WO 2004023146 A2 WO 2003-CA1354 20030905; AU 2003264211 A1 AU 2003-264211 20030905
FDT AU 2003264211 A1 Based on WO 2004023146
PRAI US 2002-408922P 20020906
IC ICM G01N033-68
AB WO2004023146 A UPAB: 20040505
NOVELTY - Identifying protein-protein interactions comprising prey proteins interacting with one or more bait comprises introducing one or more prey proteins in labeled with an epitope tag and one or more bait protein in cells labeled with a detectable substance.

DETAILED DESCRIPTION - Identifying protein-protein interactions comprising prey proteins interacting with one or more bait comprises:

(a) introducing one or more prey proteins in cells, where a prey is labeled with an epitope tag permitting separation of the prey protein from other proteins in the cells;

(b) introducing one or more bait protein in cells, where a bait protein is labeled with a detectable substance permitting detection of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;

(c) inducing formation of protein-protein interactions between a prey and bait protein; and

(d) assaying for protein-protein interactions comprising a prey protein and bait protein by detecting the detectable substance.

INDEPENDENT CLAIMS are also included for:

(1) quantitating protein-protein interactions;

(2) determining an interactome for one or more bait protein;

(3) determining the functions of gene product;

(4) systematically and quantitatively analyzing protein-protein interactions in cell signaling;

(5) determining the changes in an interactome of mitotic kinase during cell cycle progression;

(6) analyzing protein-protein interactions in different cell types;

(7) assaying for changes in protein-protein interactions in response to intracellular and extracellular factors;

(8) identifying a potential modulator of signal transduction activity; and

(9) an agent, modulator or inhibitor identified by a method of (8).

ACTIVITY - Antiinflammatory; Cytostatic.

No biological data given.

MECHANISM OF ACTION - None Given.

USE - The method and kits are useful in identifying, quantifying and analyzing protein-protein interactions. The method is useful in determining a disease or condition associated with a test protein, monitoring the course of therapy, conducting a drug discovery business and in detecting mutations in cellular proteins. The pharmaceutical composition is useful in treating and preventing a disease or condition associated with an abnormality in a signal transduction pathway, e.g. fibrosis, inflammation or cancer.

Dwg.0/3
FS CPI EPI

FA AB
MC CPI: B04-G01; B04-G21; B04-G22; B11-C07A; B11-C08E1; B11-C08F4; B12-K04A; B12-K04E; B14-C03; B14-H01; D05-H08; D05-H09; D05-H11
EPI: S03-E14H4
TECH UPTX: 20040505
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: Quantitating protein-protein interactions comprises the steps (a)-(c) of the method above and quantitating the protein-protein interactions comprising a prey and bait protein. Alternatively, the method comprises expressing one or more prey protein and bait protein in cells, obtaining a lysate of the cells and assaying an aliquot of the lysate to measure total expression of the epitope tag and detectable substance, assaying a second aliquot of the lysate to measure the amount of a detectable substance that coprecipitates with an epitope tagged prey protein and comparing the amount measured in (b) and (c) to quantitate the protein-protein interaction. The cells are subjected to an extracellular or intracellular signal after expressing the proteins.
Determining an interactome for one or more bait protein comprises preparing recombinant cells each expressing one or more bait protein and one or more prey protein from a variegated population of prey proteins, inducing formation of protein-protein interactions between a prey and bait protein in the cells and identifying protein-protein interactions comprising a prey and bait protein. Determining the functions of gene product comprises defining an interactome of the gene product using the method of (2) and determining the function of the gene product based on the structure and/or function of prey proteins that interact with the gene product in the interactome. Systematically and quantitatively analyzing protein-protein interactions in cell signaling comprises the steps of a method of identifying protein-protein interactions and comparing the types of and quantitating protein-protein interactions at the different time points.
Determining the changes in an interactome of mitotic kinase during cell cycle progression comprises introducing into the cells one or more prey protein labeled with an epitope tag permitting separation of the prey protein from other proteins in the cells and one or more mitotic kinase labeled with a detectable substance permitting identification of mitotic kinase and protein-protein interactions comprising the mitotic kinase and a prey protein, assaying for protein-protein interactions comprising a prey and mitotic kinase at different time points and comparing the types and kind of protein-protein interactions at different time points.
Analyzing protein-protein interactions in different cell types comprises introducing into first and second cells one or more prey protein and one or more bait protein, inducing cell signaling in first and second cells forming protein-protein interactions comprising a prey and bait protein and comparing the protein-protein interaction identified in the first and second cells. The first cells are from a subject with disease and the second cells are normal cells.
Assaying for changes in protein-protein interactions in response to intracellular and extracellular factors comprises introducing one or more prey proteins and one or more bait proteins in cells, inducing formation of protein-protein interactions between a prey and bait protein, introducing an intracellular or extracellular factor, assaying protein-protein interaction comprising a prey and bait protein and comparing the assayed protein-proteins interactions in the absence of intracellular or extracellular factors.
Identifying a potential modulator of signal transduction activity comprises introducing one or more prey protein and one or more bait proteins in cells, introducing a test agent in the cell, inducing formation of protein-protein between a prey and bait protein, assaying protein-protein interaction comprising a prey ad bait protein and comparing the assayed protein-proteins interactions in the absence of a test agent to determine the effect of the agent on the protein-protein interactions, where a change in the protein-protein interactions indicates

that the test agent is a potential modulator. An increase and decrease in the protein-protein interactions respectively indicates that the agent is an agonist or antagonist. The cells are mammalian cells. One or more bait and prey proteins is introduced or expressed in the cells. The detectable substance is an enzyme, radioisotope, fluorescent label, luminescent label, preferably an enzymatic label, i.e. luciferase, specifically Renilla luciferase. The epitope tag is FLAG, hemagglutinin, His6 or an Ig sequence. The prey protein comprises a protein sequence obtained from genomic DNA sequences or random sequences or a library of protein sequences. The bait protein is a functional domain of a protein involved in signal transduction. The bait protein is a protein of the TGFbeta proteome, Wnt/Wingless pathway, Sak/Polo pathway or a receptor tyrosinase kinase pathway. The bait protein is a Smad protein, SARA family protein, Smad-interacting protein, TGF beta receptor, TGF beta receptor interacting protein, **SMURF**, BMP receptor, APC, beta-catenin, axin, disheveled, GSK-3 beta, TCFs1-4, Sak, Plks, EGF, FGF, PDGF or NGF. Protein-protein interactions are assayed by purifying prey protein and complexes comprising the prey protein based on epitope tag and co-purifying the protein-protein interactions comprising the prey and bait protein by detecting the detectable substance. The prey and bait protein and complexes are purified by immunoprecipitation with an antibody specific for the epitope tag.

ABEX UPTX: 20040505

WIDER DISCLOSURE - (1) Constructing a protein linkage map for a proteome or interactome; and
(2) an integrated modular system for performing the methods.

ADMINISTRATION - Administration is by oral, subcutaneous, intravenous, intraperitoneal, intranasal, enteral, topical, sublingual, intramuscular, intraarterial, intramedullary intrathecal, inhalation, transdermal or rectal means. No dosage given.

EXAMPLE - No relevant example given.

L46 ANSWER 3 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2004-247973 [23] WPIX
DNN N2004-196735 DNC C2004-096855
TI Diagnosing glioma by detecting expression product of any one of 255 genes, glioma endothelial markers, in brain tissue sample suspected of being neoplastic, and comparing the expression with expression in normal brain tissue sample.
DC B04 D16 S03
IN COOK, B P; LATTERA, J; MADDEN, S I; WALTER, K; WANG, C J
PA (GENZ) GENZYME CORP; (UYJO) UNIV JOHNS HOPKINS
CYC 105
PI WO 2004016758 A2 20040226 (200423)* EN 114 C12N000-00
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
AU 2003262717 A1 20040303 (200457) C12N000-00
ADT WO 2004016758 A2 WO 2003-US25614 20030815; AU 2003262717 A1 AU 2003-262717 20030815
FDT AU 2003262717 A1 Based on WO 2004016758
PRAI US 2003-458978P 20030401; US 2002-403390P 20020815
IC ICM C12N000-00
AB WO2004016758 A UPAB: 20040405
NOVELTY - Aiding in diagnosis of glioma involves detecting expression product of at least one gene (I) in first brain tissue sample (T) suspected of being neoplastic, where (I) is chosen from any one of 255

genes (glioma endothelial markers (GEMs)) as given in specification, and comparing expression of (I) in (T) with expression of (I) in second normal brain tissue sample (R), where increased expression of (I) in (T) relative to (R), identifies (T) as likely to be neoplastic.

DETAILED DESCRIPTION - Aiding (M1) in diagnosing glioma involves detecting an expression product of at least one gene (I) in a first brain tissue sample suspected of being neoplastic, where (I) is chosen from any one of 255 genes (glioma endothelial markers (GEMs)) as given in specification, e.g., signal sequence receptor, delta (translocon-associated protein delta); DC2 protein; KIAA0404 protein; symplekin; Huntingtin interacting protein I; plasmalemma vesicle associated protein; KIAA0726 gene product; latexin protein; transforming growth factor, beta 1; hypothetical protein FLJ22215; Rag C protein; hypothetical protein FLJ23471; N-myristoyltransferase 1; hypothetical protein dJ1181N3.1; ribosomal protein L27; secreted protein, acidic, cysteine-rich (osteonectin); Hs 111988; Hs 112238; laminin, alpha 5; protective protein for beta -galactosidase (galactosialidosis); Melanoma associated gene; E3 ubiquitin ligase **SMURF1**; collagen, type IV, alpha 1; insulin-like growth factor binding protein 7; gene predicted from cDNA with a complete coding sequence; Thy-1 cell surface antigen; Hs127824; GTP binding protein 2; Homo sapiens mRNA; cDNA DKFZp586D0918 (from clone DKFZp586D0918); cutaneous T-cell lymphoma-associated tumor antigen se20-4; differentially expressed nucleolar TGF- beta 1 target protein (DENTT); dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive); smoothelin; integrin, alpha 5 (fibronectin receptor, alpha polypeptide); putative translation initiation factor; retinoic acid induced 14; matrix metalloproteinase 9 (gelatinase B, 95 kD gelatinase, 92 kD type IV collagenase); Lutheran blood group (Auberger b antigen included); stanniocalcin 2; nuclear factor (erythroid-derived 2)-like 2; protein tyrosine phosphatase, non-receptor type 1; etc; and comparing expression of (I) in (T) with expression of (I) in second normal brain tissue sample (R), where increased expression of (I) in (T) relative to (R), identifies (T) as likely to be neoplastic. The method optionally involves detecting mRNA of at least one gene in (T), where the at least one gene is identified by a tag which has a sequence of AAACCATTCT, AAGGCAGGGA, ACACAGCAAG, AGCTGGAGTC, AGCTGGCACC, ATAAATGAGG, CAAGCACCCC, CACTACCCAC, CACTACTCAC, CCCACCTCCA, CCCGCCTCTT, CCTCAGATGT, CGCTACTCAC, CTAAGACCTC, CTAAGACTTC, GAGTGGGTGC, GGGACAGCTG, GGGTTGGCTT, GTAAGTGTAC, GTAAGTGTAC, GTAGGGGTAA, TAACCACTGC, TACTGCTCGG, TCAGGCTGAA, TCCATACACC, TCCTTTTAAA, TGATTAAGGT, TGGTATCACA, TGGTGTATGC, TGTCACTGGG, TGTGGGAGGC, or TTTAACGGCC (S1-S32), and comparing expression of the at least one gene in (T) with expression of the gene in (R), where increased expression of the gene in (T) relative to (R) identifies (T) as likely to be neoplastic.

INDEPENDENT CLAIMS are also included for the following:

(1) treating (M2) glioma involves contacting cells of the glioma with an antibody that specifically binds to a extracellular epitope of protein chosen from plasmalemma vesicle associated protein; KIAA0726 gene product; osteonectin; laminin, alpha 5; collagen, type IV, alpha 1; insulin-like growth factor binding protein 7; Thy-1 cell surface antigen; dysferlin, limb girdle muscular dystrophy 2B; integrin, alpha 5; matrix metalloproteinase 9; Lutheran blood group, integrin, alpha 10, collagen, type VI alpha 2; glioma endothelial marker 1 precursor; translocase of inner mitochondrial membrane 17 homolog A; heparan sulfate proteoglycan 2; annexin A2; matrixmetalloproteinase 10; G protein-coupled receptor; matrix metalloproteinase 14; solute carrier family 29, member 1; CD59 antigen p18-20; KIAA 1870 protein; plexin B2; lectin; integrin beta 4 binding protein; acetyl low density lipoprotein (LDL) receptor; laminin, gamma 3; macrophage migration inhibitory factor; gap junction protein, alpha 1, 43 kD; aquaporin 1; protease, serine, 11; collagen, type IV, alpha 2; apolipoprotein D; plasminogen activator; urokinase; insulin-like growth factor binding protein 3; regulator of G-protein signaling 12; prosaposin; etc.;

(2) identifying (M3) a test compound as potential anticancer or

antiglioma drug involves contacting a test compound with the cell which expresses (I), monitoring an expression product of the at least one gene and identifying test compound as a potential anticancer drug if it decreases the expression of at least one gene;

(3) identifying (M4) a test compound as potential anticancer or antiglioma drug involves contacting a test compound with the cell which expresses mRNA of at least one gene identified by a tag as described above, monitoring mRNA of the gene, and identifying the test compound as a potential anticancer drug if it decreases the expression of at least one gene; and

(4) inducing (M5) an immune response to glioma involves administering to a mammal, a protein or (I).

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Triggers immune destruction of glioma cells; Immune response inducer. No supporting data is given.

USE - (M1) is useful for aiding in diagnosing glioma. (M2) is useful for treating multi-drug sensitive glioma in a human. (M5) is useful for inducing an immune response to a glioma in a mammal having glioma or in a mammal who has had a glioma surgically removed (claimed).

Dwg.0/0

FS CPI EPI

FA AB; DCN

MC CPI: B04-E03E; B04-E03F; B04-E12; B04-F02; B04-F02A; B04-G01; B04-H01; B04-H06F; B04-L01; B04-N04; B04-N06; B11-C07A; B11-C07A3; B11-C08E3; B11-C08E5; B11-C08E6; B12-K04A1; B12-K04E; B12-K04F; B14-H01; D05-H08; D05-H09; D05-H11; D05-H12A

EPI: S03-E14H4

TECH UPTX: 20040405

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: Increased expression of (I) in (T) relative to (R) is at least two-fold higher, preferably at least 10-fold higher. The expression product is RNA or protein, where the protein is detected using Western blot or by an immunoassay or immunohistochemical assay, and the RNA is detected using serial analysis of gene expression (SAGE), or by using hybridization to microarray. (T) and (R) are from a human, preferably same human. Detecting In (M2), the antibody is conjugated to a diagnostic or therapeutic agent, e.g., chemotherapeutic agent, cytotoxin, a nonradioactive label, or radioactive compound. In (M3) or (M4), the test compound is contacted with the human glioma cell, where the cell overexpresses at least one gene relative to normal cell of the same tissue. The expression product is a protein or RNA. The method involves monitoring expression of at least two of the genes, preferably four of the genes. The test compound is identified if the decrease in expression is at least 50%, preferably 90%. The test compound is identified as an antiglioma drug. (M5) further involves administering an immune adjuvant to the mammal.

ABEX UPTX: 20040405

WIDER DISCLOSURE - Use of glioma endothelial markers for identifying endothelial cells, and for stimulating the growth of vasculature, such as for wound healing, or to circumvent a blocked vessel, is also disclosed.

ADMINISTRATION - The nucleic acid is administered intramuscularly (claimed). The proteins and nucleic acids are administered by parenteral, intravenous, intraperitoneal, topical, intranasal, intrarectal or intrabronchial route.

EXAMPLE - Five separate brain tissue samples were resected and immediately subjected to endothelial cell isolation. Briefly, samples were surgically excised and submerged in Dulbeccos' modified Eagle's medium (DMEM). The samples were minced into 2 cm3 and subjected to tissue digestion with a collagenase cocktail. Samples were mixed at 37 degrees C until dissolved. Cells were spun down and washed two times with phosphate buffered saline/bovine serum albumin (PBS/BSA) and filtered through successive nylon mesh filters of 250, 100 and 40 microns. Samples were resuspended in PBS/BSA and applied to a 30% Percoll gradient centrifugation. Five ml off

the top of the Percoll gradient was diluted in 50 ml DMEM and cells pelleted, washed with PBS and resuspended in 3 ml PBS/BSA. Cells were filtered through falcon blue top filter tubes, spun down and resuspended in 1 ml PBS/BSA. 100 microl of prewashed ant-CD45 magnetic beads were added and the solution allowed to gently mix for ten minutes. Bead-bound cells were discarded and the supernatant transferred to a fresh microcentrifuge tube. 10 microl of P1H12 mAB (1:100) (Brain N1, T1, and T2 samples) or UEA-I lectin (Brain N2 and T3 samples) was added and the samples were mixed gently at 4 degrees C for 45 minutes. Cells were pelleted and washed 3 times in PBS/BSA and resuspended in 500 microl PBS/BSA. Prewashed goat anti-mouse M450 Dynabeads were added to each tube and allowed to mix for 15 minutes at 4 degrees C. Bead-bound cells were washed 8 times with PBS/BSA and resuspended in a final volume of 500 microl PBS. Cells were counted and frozen at -70 degrees C prior to RNA extraction. RNA was isolated from the selected cells and initially subjected to reverse transcriptase polymerase chain reaction (RT-PCR) analysis to determine the relative abundance of specific, known endothelial cell markers. The microserial analysis of gene expression (SAGE) protocol was used to generate high-quality longSAGE libraries employing the tagging enzyme MmeI instead of BsmFI. 21 base tags were defined by capillary sequencing using a combination of an ABI 3700 and ABI 3100. Long SAGE tags derived from the brain endothelial samples were reduced to short tags to allow for the integration of colon endothelial SAGE data. Aggregate short tags were derived from the long tags. Any short tag counts that had more than one corresponding long tag representative were summed and the counts represented as one short tag. Both sequencing errors and legitimate long tag derivatives contribute to the generation of multiple long tags. For transcript and genome mapping, differential long tags were employed. Differential gene expression was evaluated as follows: For the two normal brain samples, either the maximum or minimum value was used for determining tumor/normal and normal/tumor ratios, respectively. For the three brain tumor samples, the median value was used for the tumor/normal whereas the maximum value was used for the normal/tumor ratios. A two parameter family of beta distributions was used to assess the probability of observing two fold differences in the observed SAGE tag abundances. 255 human genes were identified that were expressed at significantly higher levels in brain tumor endothelium than in normal brain endothelium. These markers were named as glioma endothelial markers (GEMs).

L46 ANSWER 4 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2003-430236 [40] WPIX
DNN N2003-343514 DNC C2003-113665
TI Treatment of bone defect conditions involves use of compound or protocol, which is inhibitory to ubiquitin ligases.
DC B04 S03
IN CHEN, D; GARRETT, I R; MUNDY, G R; ROSSINI, G; ZHAO, M
PA (CHEN-I) CHEN D; (GARR-I) GARRETT I R; (MUND-I) MUNDY G R; (ROSS-I) ROSSINI G; (ZHAO-I) ZHAO M; (OSTE-N) OSTEOSCREEN INC
CYC 101
PI WO 2003030924 A1 20030417 (200340)* EN 19 A61K038-00
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VC VN YU ZA ZM ZW
US 2003092603 A1 20030515 (200340) A61K031-00
ADT WO 2003030924 A1 WO 2002-US33615 20021009; US 2003092603 A1 Provisional US 2001-328300P 20011009, Provisional US 2002-346742P 20020107, US 2002-268374 20021009
PRAI US 2002-346742P 20020107; US 2001-328300P 20011009;

US 2002-268374 20021009
IC ICM A61K031-00; A61K038-00
ICS A61K039-00; C12Q001-68; G01N033-567
AB WO2003030924 A UPAB: 20030624
NOVELTY - Treatment of bone defect conditions involves administering to a subject, a protocol or a compound, which is inhibitory to beta -TrCP, Smad ubiquitin regulatory factor-1 (**Smurf1**) or **Smurf2**.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for identification of compound or protocol, which enhances bone formation and/or osteoblast differentiation involving assessing the ability of a candidate compound or protocol to inhibit activity of beta -TrCP, **Smurf1** or **Smurf2**.

ACTIVITY - Osteopathic; Antiarthritic; Cytostatic; Vulnerary; Antiinflammatory; Periodontal.

Test details are described but no suitable results are given.

MECHANISM OF ACTION - Bone growth stimulator or bone formation modulator; beta -TrCP, **Smurf1** and **Smurf2** inhibitor.

USE - For the treatment of bone defect conditions (claimed) (e.g. osteoporosis including age related osteoporosis, post-menopausal osteoporosis, glucocorticoid-induced osteoporosis or disuse osteoporosis and arthritis); for repair of congenital trauma-induced or surgical resection of bone e.g. cancer treatment, and in cosmetic surgery; for tooth repair; for treating cartilage defects or disorders; and in wound healing or tissue repair. Also useful for the treatment of growth deficiencies, periodontal diseases and defects.

ADVANTAGE - The compound or the protocol enhances bone formation and osteoblast differentiation. The compounds repair bone defects and deficiencies occurring in closed, open and non-union fractures, in closed and open fracture reduction, promotes bone healing in plastic surgery, stimulates bone in growth into non-cemented prosthetic joints and dental implants, elevates the peak bone mass in pre-menopausal women, increases bone formation during distraction osteogenesis. Therefore, the compounds clinically increase healing rates in fracture repair, reverse bone loss in osteoporosis, reverse cartilage defects, prevent or delay onset of osteoporosis, stimulate or augment bone formation in fracture non-unions and distract osteogenesis, increase bone growth in prosthetic device and repair dental defects. The compound stimulates growth of bone-forming cell precursors either in vitro or ex vivo and modifies a target tissue or organ environment so as to attract bone-forming cells to an environment in need of such cells, particularly the compounds stimulate a cell population containing marrow mesenchymal cell thus increasing the number of osteogenic cells in that cell population.

Dwg.0/6
FS CPI EPI
FA AB; DCN
MC CPI: B04-C01A; B04-H01; B04-L08; B07-D03; B14-C09; B14-D10; B14-E11; B14-H01; B14-N01; B14-N06B; B14-N17B
EPI: S03-E14H
ABEX UPTX: 20030624
ADMINISTRATION - The compounds are administered in a daily dosage of 0.1 - 1000 (preferably 1 - 200) mg/kg parenterally (including intravenously, subcutaneously, intramuscularly, intraperitoneally, intranasally or transdermally), enterally (including orally or rectally) or topically. The parenteral dosage is 20 - 100% of the oral dosage.
EXAMPLE - No relevant example given.

L46 ANSWER 5 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2003-120443 [11] WPIX
DNC C2003-031029
TI New breast cancer-associated (BCA) genes and polypeptides, useful for preventing, treating, diagnosing or staging breast cancer, or other BCA-related disorders, e.g. prostate cancer, sarcoma, Ewing's tumor, leukemia or lymphomas.

DC B04 D16
IN SETH, A
PA (SUNN-N) SUNNYBROOK & WOMEN'S COLLEGE HEALTH SCI
CYC 101
PI WO 2002087507 A2 20021107 (200311)* EN 195 A61K000-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
EP 1399460 A2 20040324 (200421) EN C07H021-02
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
AU 2002311869 A1 20021111 (200433) A61K000-00
ADT WO 2002087507 A2 WO 2002-US13584 20020429; EP 1399460 A2 EP 2002-739203 20020429, WO 2002-US13584 20020429; AU 2002311869 A1 AU 2002-311869 20020429
FDT EP 1399460 A2 Based on WO 2002087507; AU 2002311869 A1 Based on WO 2002087507
PRAI US 2001-287170P 20010427
IC ICM A61K000-00; C07H021-02
ICS C12N005-12; C12N015-63; C12P021-06
AB WO 200287507 A UPAB: 20030214
NOVELTY - An isolated polynucleotide, which comprises a breast cancer-associated (BCA) gene or a polynucleotide sequence encoding a chimeric protein, is new. The polynucleotide consists of the human BCA1, BCA2, BCA3, BCA4, BCA5, BCA6 or BCA7 gene.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) A human BCA polypeptide encoded by the polynucleotide;

(2) Fragments of the human BCA polypeptide;

(3) A complex comprising BCA1 polypeptide and a binding partner consisting of a gene product of AIP4, **Smurf2**, polyubiquitin UbC, DUT, EPS15, ZBRK1, chromosome 10 open reading frame 5, AMSH, PLAT, TOM1L2, FLJ11626, clone 155, VIM, INVS, clone 287, clone 292 or POLR2J;

(4) An antibody that immunospecifically binds to a human BCA1, BCA2, BCA3, BCA4, BCA5, BCA6 or BCA7 polypeptide;

(5) An expression vector comprising the human BCA polynucleotide;

(6) A cell comprising a recombinant human BCA1, BCA2, BCA3, BCA4, BCA5, BCA6 or BCA7 polynucleotide;

(7) A transgenic non-human animal comprising a transgene that comprising the human BCA1, BCA2, BCA3, BCA4, BCA5, BCA6 or BCA7 polynucleotide;

(8) Diagnosing (M1) and staging a BCA-related disorder in a subject;

(9) Identifying (M2) an analyte that binds to the BCA polypeptide;

(10) Identifying (M3) a protein that binds the BCA polypeptide;

(11) Identifying (M4) an analyte that binds a complex comprising the BCA polynucleotide or polypeptide;

(12) Identifying (M5) an analyte that inhibits formation of a complex comprising the BCA polynucleotide or polypeptide;

(13) Identifying (M6) an inhibitor of growth of a breast cancer cell; and

(14) A kit comprising:

(a) a first container with a purified BCA nucleic acid, BCA polypeptide, BCA agonist, or BCA antagonist; and

(b) a second container with a molecule that binds to the BCA nucleic acid, BCA polypeptide, BCA agonist or BCA antagonist when bound to an analyte in a biological sample.

ACTIVITY - Cytostatic; Immunostimulant; Antiallergic; Osteopathic; Anabolic.

No biological data given.

MECHANISM OF ACTION - Gene Therapy; Vaccine.
No biological data given.
USE - The BCA polynucleotide, BCA polypeptide, anti-BCA polypeptide antibody, expression vector or antisense BCA polynucleotide is useful for preventing or treating breast cancer. These are also useful for diagnosing or staging a BCA-related disorder such as breast cancer (all claimed). Other BCA-related disorders that may be treated with the BCA polynucleotide or polypeptide or antibody are allergy, bone disease, eating disorder, infectious disease, ovarian cancer, prostate cancer, skin cancer or brain cancer, malignant or non-malignant tumors, sarcoma, Ewing's tumor, leukemia, lymphomas, or polycythemia vera. The BCA polynucleotide and polypeptide are also useful in forensic biology, diagnostic assays, prognostic assays or pharmacogenomics, or for monitoring clinical trials.
Dwg.0/22

FS CPI
FA AB; DCN
MC CPI: B04-C01A; B04-C01G; B04-E01; B04-F0100E; B04-G01; B04-G05; B04-N02A0E; B04-P0100E; B11-C07A; B11-C08E; B12-K04A1; B12-K04E; B14-E11; B14-G01; B14-G02A; B14-H01; B14-N01; B14-S03; B14-S11C; D05-C12; D05-H09; D05-H11; D05-H12A; D05-H12B1; D05-H12D; D05-H12E; D05-H14B; D05-H16A; D05-H17A6; D05-H17B6
TECH UPTX: 20030214
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Polynucleotide: The BCA polynucleotide comprises:
(a) a nucleotide sequence of the human BCA3 gene, which comprises 1319 bp fully defined in the specification;
(b) a nucleotide sequence that encodes a human BCA3 polypeptide;
(c) a nucleotide sequence that encodes the BCA3 polypeptide having 210 amino acids fully defined in the specification, or having the following sequence: Gly-Gly-Pro-Gly-Gly;
(d) at least 12 consecutive bases of the human BCA3 gene, where the polynucleotide is not F29989, BG754249, BG654786, AU146189, AU145473, AV729000, AV725974, BE349302, BE205860, AW406755, AW339687, AI635272, AI365988, BM469324, BM558580, AW510839, BF337353, AA640772, AL599210, AL571890, BF913170, BE149796, BG681808, AA478355, BE304890, BI058894, BM042507, BG773327, AA521399, AA521323, AI873852, BI030630, BI023028, BG819532, BE909262, BE293845, BE293802, AW675725, AW193295, F19258, AI358229, AA478297, BG566176, AJ400877, NM 020642 Nor BM449949; or
(e) a nucleotide sequence that is a complement of (d).
The polynucleotide comprises a nucleotide sequence of 640 bases in length that hybridizes under highly stringent conditions to:
(a) a nucleotide sequence complementary to the coding region of the human BCA3 gene; or
(b) the nucleotide sequence of a human BCA3 mRNA.
The isolated polynucleotide comprises a nucleotide sequence encoding a fragment of the human BCA3 protein, where the fragment displays one or more functional activities. The isolated polynucleotide comprises a BCA1 nucleotide sequence, which comprises residues 1-2659, 1-2500, 1-2000, 1-1500, 1-1000, 1-500, 1-124, 2516-2659, 2500-2659, 2000-2659, 1500-2659, 1000-2659, 500-2659, 124-2659, 363-377, or 551-674 of a 2659-bp sequence fully defined in the specification, or a 123-bp sequence or 19-bp sequence fully defined in the specification. The isolated polynucleotide also comprises a BCA2 nucleotide sequence consisting of residues 1-2176, 1-2000, 1-1500, 1-1000, 1-500, 1-100, 2000-2176, 1500-2176, 1000-2176, 500-2176, 100-2176, 768-782 or 980-1052 of a 2177-bp sequence fully defined in the specification, or a sequence having 15, 20, 15, 15 or 300 bp fully defined in the specification.
The polynucleotide also comprises a nucleotide sequence of at least 12 consecutive bases encoding a portion of a domain of:
(a) a human BCA1 polynucleotide or polypeptide, where the domain is RING H2, finger, PY motif, glycosylation site, phosphorylation site, SH2-binding motif, open-reading frame, exon 1, exon 2, exon 3, intron 1,

intron 2, 5' untranslated region, or 3' untranslated region;
(b) a human BCA2 polynucleotide or polypeptide, where the domain is RING H2, NPXXY motif, PXXP motif, zinc finger, glycosylation site, phosphorylation site, SH3-binding motif, open-reading frame, exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, exon 9, intron 1, intron 2, intron 3, intron 4, intron 5, intron 6, intron 7, intron 8, 5' untranslated region, or 3' untranslated region; or
(c) a human BCA3 polynucleotide or polypeptide, where the domain is SH2 site YYSS, SH2 site YSSV, SH2 site YHRG, SH2 site YIEV, SH2 site YPGT, SH2 site YSVT, tyrosine phosphorylation site, RTMAEFMDY, glycosylation site, phosphorylation site, tyrosine phosphorylation motif, SH2-binding motif, open-reading frame, open-reading frame lacking exon 3, open reading frame lacking exon 3 and exon 5, exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, intron 1, intron 2, intron 3, intron 4, intron 5, intron 6, 5' untranslated region, or 3' untranslated region.
The polynucleotide encoding the human BCA3 polypeptide is an RNA.
Preferred Polypeptide: The human BCA3, BCA1 and BCA2 comprise a sequence having 210, 154 or 304 amino acids, respectively. These sequences are fully defined in the specification. The fragment of the human BCA1, BCA2 or BCA3 polypeptide comprises at least 5 consecutive amino acids of the human BCA1, BCA2 and BCA3 polypeptide, respectively. The fragment is a portion of the domains cited above. The polypeptide has an amino acid sequence that has at least 90% identity to the fragment described above, or to the amino acid sequences cited above.
Preferred Antibody: The antibody immunospecifically binds to a human BCA polypeptide when bound to a binding partner.
Preferred Methods:
M1 comprises:
(a) contacting a BCA antibody with a sample suspected of containing a BCA polypeptide from the subject, under conditions that allow the BCA antibody to bind the BCA polypeptide; and
(b) detecting or measuring binding of the BCA antibody to the BCA1, BCA2, BCA3, BCA4, BCA5, BCA6 or BCA7 polypeptide.
The BCA-related disorder is determined to be present when the presence or amount of the BCA polypeptide indicated by the detection or measurement of binding differs from a control value representing the amount of BCA polypeptide present in an analogous sample from a subject not having the BCA-related disorder. The stage of a BCA-related disorder in a subject is determined when the presence or amount of BCA polypeptide indicated by the detection or measurement of binding is compared with the amount of BCA polypeptide present in an analogous sample from a subject having a particular stage of a BCA-related disorder, e.g. breast cancer.
M2 comprises:
(a) contacting the BCA polypeptide with an analyte to allow the analyte to bind the BCA polypeptide; and
(b) detecting binding of the BCA polypeptide to the analyte.
M3 comprises:
(a) contacting the BCA polypeptide with a positionally addressable array comprising several proteins, with each protein at a different position on a solid support; and
(b) detecting binding of the BCA polypeptide to a protein on the array.
M4 comprises:
(a) contacting the complex with the analyte to allow the analyte to bind to the complex; and
(b) detecting binding of the BCA polynucleotide or polypeptide to the analyte, where the analyte binds to the BCA polynucleotide or polypeptide when bound to the binding partner, and does not bind to the BCA polynucleotide or polypeptide when not bound to the binding partner.
M5 identifying an analyte that inhibits formation of a complex comprising the BCA polynucleotide or polypeptide comprises:
(a) contacting the complex with the analyte; and
(b) measuring the amount of the complex, where a reduction in the amount of complex indicates that the analyte inhibits formation of the complex.

M6 comprises:
(a) contacting the cell with: (a.1) the BCA polynucleotide; (a.2) the BCA polypeptide; or (a.3) the antibody that immunospecifically binds to the BCA polypeptide; and
(b) measuring cell growth, where an inhibition of cell growth indicates the presence of an inhibitor of growth of a breast cancer cell.
Preparation: The BRA-3 polypeptide is prepared by standard recombinant techniques comprising culturing a cell expressing BRA-3 polynucleotide and isolating the polypeptide (claimed). The polynucleotide, expression vector, cell and transgenic animal are prepared by standard recombinant techniques.

ABEX UPTX: 20030214

SPECIFIC SEQUENCES - Specifically claimed is a human BCA polypeptide comprising sequences of, for example 210 (BCA3), 154 (BCA1) and 304 (BCA2) amino acids, fully defined in the specification. Also claimed are nucleic acid sequences for example, the nucleotide sequence of the human BCA3 gene, which comprises 1319 bp fully defined in the specification.

ADMINISTRATION - Dosage is 0.01-10 mg/kg/day. Preferably, dosage is 0.01-5 or 10-50 mg/kg, depending on the route of administration. Administration is oral, intravenous, subcutaneous, transdermal, rectal, intramuscular, topical, depo injection, implantation, time-release mode, intracavitary, intranasal, intratumoral, intraocular, or parenteral.

EXAMPLE - More than 1000 cDNA clones for genes that may be activated or inactivated during progression of breast cancer were isolated by subtractive hybridization and differential display methods using matched breast tumor and normal breast cell line RNAs. All cDNA sequences from the breast cancer subtractive cloning library were compared by BLAST to the entire-redundant GenBank database. Seven genes, namely BCA1-7, were identified. Two genes, BCA1 and BCA3 were expressed more highly in abreast tumor tissue than in normal breast tissue.

L46 ANSWER 6 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2001-071267 [08] WPIX
DNC C2001-019969
TI Novel isolated **Smurf** protein useful for inhibiting bone morphogenic protein or tumor growth factor-beta activation pathway, for treating cancer and to block osteogenesis, hair growth, tooth formation.
DC B04 D16
IN THOMSEN, G H; WRANA, J
PA (HSCR-N) HSC RES & DEV LP; (UYNY) UNIV NEW YORK STATE RES FOUND
CYC 93
PI WO 2000077168 A2 20001221 (200108)* EN 106 C12N000-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000056107 A 20010102 (200121)
EP 1192174 A2 20020403 (200230) EN C07H021-04
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
JP 2003502064 W 20030121 (200308) 131 C12N015-09
CN 1409722 A 20030409 (200345) C07H021-04
ADT WO 2000077168 A2 WO 2000-US16250 20000612; AU 2000056107 A AU 2000-56107 20000612; EP 1192174 A2 EP 2000-941398 20000612, WO 2000-US16250 20000612; JP 2003502064 W WO 2000-US16250 20000612, JP 2001-504003 20000612; CN 1409722 A CN 2000-811354 20000612
FDT AU 2000056107 A Based on WO 2000077168; EP 1192174 A2 Based on WO 2000077168; JP 2003502064 W Based on WO 2000077168
PRAI US 1999-138969P 19990611

IC ICM C07H021-04; C12N000-00; C12N015-09
ICS A01K067-027; A61K031-7088; A61K038-00; A61K039-395; A61K045-00; A61K048-00; A61P019-08; A61P025-00; A61P027-02; A61P043-00; C07K014-00; C07K016-40; C12N001-15; C12N001-19; C12N001-21; C12N005-10; C12N009-00; C12N015-00; C12Q001-02; G01N033-15; G01N033-50; G01N033-53

AB WO 200077168 A UPAB: 20011129
NOVELTY - An isolated **Smurf1** or **Smurf2** protein (I), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated nucleic acid (II) encoding (I);
(2) a vector (III) comprising (II);
(3) a host cell (IV) comprising (III);
(4) production of (I);
(5) a transgenic non-human animal that expresses a human (I);
(6) screening (M) for a modulator of **Smurf** activity, comprising detecting modulation of **Smurf** activity in the presence of a test compound relative to **Smurf** activity in the absence of the test compound;
(7) an antibody (V) that specifically binds to (I);
(8) an oligonucleotide or nucleic acid (VI) that specifically hybridizes to (II) under highly stringent conditions; and
(9) promoting a bone morphogenic protein or transforming growth factor (TGF)- beta activation pathway in a cell, comprising suppressing expression of endogenous **Smurf** in the cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Negative regulator of Smad signal transduction; antagonist of BMP and TGF- beta signaling pathway.

The inhibition of Smad1 by **Smurf1** was tested. By over expressing Smad1 and Smad2 together with various dosages of **Smurf1** in Xenopus animal caps, the ability of **Smurf1** to directly antagonize the mesoderm induction activities of Smad1 and Smad2, was tested. The results showed that expression of Smad1 alone induced ventral mesoderm, as demonstrated by expression of the ventral/posterior mesodermal markers Xhox3 and Xcad1. However, co-expression of **Smurf1** and Smad1 blocked induction of these markers at all **Smurf1** doses tested, demonstrating that **Smurf1** can antagonize Smad1 activity.

USE - Expression of (I) from (III) in a cell is useful for inhibiting a bone morphogenic protein (BMP) or transforming growth factor- beta (TGF beta) activation pathway in a cell (claimed). (I) is useful to block chondrogenesis, osteogenesis, blood differentiation, cartilage formation, neural tube patterning, retinal development, heart induction and morphogenesis, hair growth, tooth formation, gamete formation and a wide variety of tissue and organ formation processes, and hinder the regeneration, growth, maintenance, etc., of bone and other tissues that are dependent on the BMP pathway. (I) is useful for screening for various drugs and/or antibodies that can either enhance the BMP pathway, or inhibit it by antagonizing or mimicking the activity of (I), respectively, and in screening assays for identifying specific ligands of (I). (I) is useful as an immunogen to generate antibodies that are useful to alter the BMP pathway by inhibiting (I) or for diagnostic purposes. (I) is useful for treating a disorder associated with BMP or TGF- beta activation, such as cancer. (I) or inhibitor of (I) can be delivered by a vector to modulate Smads, e.g. to prevent **Smurf** regulation of Smads where BMP or TGF beta activity is desired, such as in bone regeneration or to study **Smurf** regulator processes in vivo.

Dwg.0/18

FS CPI
FA AB; DCN
MC CPI: B04-C01G; B04-E02F; B04-E03F; B04-E05; B04-E06; B04-E08; B04-F0100E; B04-G01; B04-N02A0E; B04-P0100E; B11-C08; B12-K04E; B14-H01;

B14-H01B; D05-C12; D05-H09; D05-H12A; D05-H12B2; D05-H12D1; D05-H12D2; D05-H12E; D05-H14; D05-H14B; D05-H16A; D05-H17A6; D05-H17B6

TECH UPTX: 20010207

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation: (I) is produced by growing (IV) under conditions that permit expression of (I) from (III) (claimed). Preferred Protein: (I) is human **Smurf1** or **Smurf2** protein and has a mutation corresponding to C710A or C716A, respectively. Preferred Method: In (M) the **Smurf** activity is ubiquitination of a Smad protein in a host cell or interaction of a **Smurf** WW domain with a PPXY domain of a Smad protein. The test compound is screened for the ability to inhibit the interaction.

ABEX UPTX: 20010207

WIDER DISCLOSURE - Disclosed as new are the following:
(1) an antisense nucleic acid which may be used to inhibit expression of **Smurf1** or **Smurf2**, particularly to enhance bone morphogenic protein or TGF-beta signaling pathway;
(2) analogs, derivatives of (I), homologs from other species, and mutant variants, which have the same or a homologous functional activity, and their production and use; and
(3) cloning vectors containing genes encoding analogs and derivatives of (I).

SPECIFIC SEQUENCES - (I) comprises at least 10 contiguous residues of a 723 or 748 residue amino acid sequence corresponding to **Smurf1** and **Smurf2**, respectively, and is encoded by (II) with a 2172 or 2247 base pair sequence, all fully defined in the specification (claimed).

EXAMPLE - A Xenopus Smad1 cDNA was cloned into the pGBT9 vector and used to screen a Xenopus oocyte cDNA library using Xenopus Smad1 as the bait protein. A partial cDNA was isolated and used to screen a Xenopus Stage9 cDNA library to obtain a full length **Smurf1** cDNA with a 2172 base pair sequence, fully defined in the specification. A human **Smurf1** cDNA encoding all but the first 8 amino acids was identified and used to construct human **Smurf1.** Human **Smurf2** was identified and cloned using a Xenopus **Smurf1** sequence. Two overlapping expressed sequence tags (EST) clones corresponding to **hSmurf2** were obtained and used to construct a full length sequence for **hSmurf2. Smurf2** was closely related to **Smurf1**, and displayed 75 % homology to the amino acid sequence of **hSmurf1.** Several overlapping human clones displaying similarity to **Smurf1** were identified and a full-length version of **Smurf2** was constructed. A partial mouse **Smurf2** cDNA clone encoding 225 amino acids of open reading frame including the stop codon and displaying 96 % amino acid identity to human **Smurf2** was identified. For mammalian expression constructs of **Smurf2**, the open reading frame was amplified and was subcloned into pCMV5 in frame with an amino-terminal Flag or Myc tag.

L46 ANSWER 7 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2000-317970 [27] WPIX
DNC C2000-096321
TI Targeting degradation of polypeptide useful for treating cancer and other proliferative disorders, involves conjugating polypeptide with ubiquitin protein ligase or inhibiting ubiquitination using organic compound.
DC B04 D16
IN HOWLEY, P; ZHOU, P
PA (HARD) HARVARD COLLEGE
CYC 87
PI WO 2000022110 A2 20000420 (200027)* EN 185 C12N015-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB

GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU
LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM
TR TT TZ UA UG UZ VN YU ZA ZW

AU 2000012030 A 20000501 (200036) C12N015-00

ADT WO 2000022110 A2 WO 1999-US23705 19991008; AU 2000012030 A AU 2000-12030 19991008

FDT AU 2000012030 A Based on WO 2000022110

PRAI US 1998-103787P 19981009

IC ICM C12N015-00
ICS C12N005-10; C12N015-12; C12N015-37; C12N015-52; C12N015-62

ICA C07K014-00

AB WO 200022110 A UPAB: 20000606

NOVELTY - Targeting degradation of a target polypeptide (I) in vivo, comprising ubiquitinating (I) by expressing in a cell, a ubiquitin protein ligase polypeptide (UL), having ubiquitin conjugation activity linked to interaction domain of (I), and recruiting (I) to (UL), is new. The ubiquitin-(I) conjugate formed is targeted for degradation.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method for decreasing the level of (I), comprising providing an SCF (SKP1, Cullin and F-box containing proteins) recruitment domain operably linked to a (I) interacting domain, to form a fusion protein, and expressing the fusion protein, so that the level of (I) is decreased;

(2) a method for creating a destabilized polypeptide subject to SCF-mediated proteolysis, comprising providing an SCF recruitment domain, and operably linking it to the polypeptide;

(3) a method for expressing a destabilized (I) subject to SCF-mediated proteolysis, comprising providing an SCF recruitment domain operably linked to (I), and expressing the fusion polypeptide;

(4) a nucleic acid (III) for expressing an SCF recruitment domain-interaction domain of (I), comprising a nucleic acid encoding an SCF recruitment domain, and a heterologous polypeptide domain;

(5) a vector (IV) comprising (III);

(6) a cell comprising (IV); and

(7) a method of treating a cell to stabilize a target of ubiquitin protein ligase, comprising contacting the cell with a preparation comprising an organic compound, which can competitively inhibit interaction of the target polypeptide with the ligase.

ACTIVITY - Cytostatic; nootropic; anticonvulsant; antimicrobial. Targeted degradation of endogenous p107 in mammalian cells was tested using beta TrCP-E7N. p107 is a protein related to the retinoblastoma tumor suppressor protein pRB. Cervical carcinoma C33A cells lacking wild type pRB were transmitted with engineered beta TrCP-E7N. The cells were co-transfected with cytomegalovirus (CMV)-CD19 and selected by immunomagnetic selection of cells expressing CD19. The results showed that levels of p107 was significantly decreased in beta TrCP-E7N expressing cells but were not affected in cells expressing the control protein unable to bind p107.

MECHANISM OF ACTION - Ubiquitin-conjugation-regulator; gene therapy.

USE - The methods are useful for decreasing or increasing the level of (I), and for creating and expressing a destabilized (I) which is subjected to SCF mediated proteolysis (claimed). Degrading any desired protein in a cell is useful for preventing or treating diseases caused by the presence of abnormal amount of the specific polypeptides, for drug discovery and for gene therapy. Diseases treated include cancer, by degradation of oncoproteins, Huntington's disease, other proliferative disorders and microbial infections.

ADVANTAGE - The method provides a quick, easy and economic alternative to gene knockout technology. (I) can be degraded at all stages, or a specific stage, of development in the mature animal.

Dwg.0/0

FS CPI

FA AB; DCN

MC CPI: B04-A08C2E; B04-E02F; B04-E08; B04-F0100E; B04-P01A0E; B11-C08E1; B11-C09; B14-A01; B14-A02; B14-A03; B14-A04; B14-H01B; B14-J01A4; B14-J07; B14-S03; D05-H09; D05-H12C; D05-H12E; D05-H14; D05-H16; D05-H17C

TECH UPTX: 20000606

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Polypeptide: The ubiquitin-target polypeptide conjugate further undergoes ubiquitin-dependent proteolysis by proteasomes. (UL) is an E3 ubiquitin protein ligase, preferably an SCF, HECT or a UBR1 polypeptide. The SCF polypeptide is selected from Skp1 or a cullin polypeptide or an F-box polypeptide, preferably Cdc4p, Pop1p, Pop 2p, Grr1p, Met30p, HOSp, beta TrCPp or FWD1p which further comprises a WD domain. The HECT polypeptide is E6-Ap, Nedd-4, RSP5, **Smurf1**, TOM1 or EDD. E3 ubiquitin protein ligase is a yeast or a mammalian UBR1 polypeptide. (I) is a retinoblastoma, a p107, IkappaB, Sic1p, Cln2p, E2 or beta catenin polypeptide. The interaction domain of (I) is either a papillomavirus E7 or a SV40 LTP polypeptide. (II) is a peptide or a peptidomimetric which is a competitive inhibitor of a WD domain comprising a general chemical formula: G-H-X(3-6)-h-X-X-h-X-r-X-t(2-3)-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D.

ABEX UPTX: 20000606

WIDER DISCLOSURE - The following are disclosed as new:
(1) transgenic plants and animals comprising (III);
(2) a kit comprising (III); and
(3) methods and compositions for the identification of inhibitors of the interaction between an F-box protein and other subunits of an SCF complex.

SPECIFIC SEQUENCES - The F-box polypeptide has a 721, 541, 1141, 601, 661 or 541 residue amino acid sequence, encoded by a 20341(Cdc4p), 2101(hbetaTrCp), 4441(Grr1p), 33901(Met30p), 2101(Pop2) or 2161(FWD1p) base pair sequence (claimed). All sequences fully defined in the specification.

ADMINISTRATION - The administration is oral, buccal, parenteral, rectal, systemic, topical or local routes. No specific dosage is given.

=> d his

(FILE 'HOME' ENTERED AT 09:44:07 ON 21 SEP 2004)
SET COST OFF

FILE 'REGISTRY' ENTERED AT 09:44:26 ON 21 SEP 2004
E SMURF
L1 20 S E3-E5 OR ?SMURF?/CNS
E SMAD
L2 403 S E3-E21

FILE 'HCAPLUS' ENTERED AT 09:46:07 ON 21 SEP 2004
L3 15 S L1
L4 85 S ?SMURF?
L5 90 S L3,L4
L6 9 S L5 AND L2
L7 48 S L5 AND ?SMAD?
L8 50 S L6,L7
L9 42 S L8 AND UBIQUITIN?
L10 50 S L8,L9
L11 7 S L10 AND SCREEN?
E DRUG SCREENING/CT
L12 24987 S E3-E5
L13 6373 S E9,E10
E E3+ALL
L14 31124 S E9,E8
E E12+ALL

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L15         9001 S E10
L16         3897 S E21+NT
L17            5 S L10 AND L12-L16
L18            7 S L11,L17
L19            8 S L10 AND ?MODULAT?
L20           11 S L5 AND ?MODULAT?
L21           15 S L18-L20
L22            0 S L10 AND ?PPYX?
L23            7 S L10 AND WW (L) DOMAIN
L24           21 S L21,L23
                 E WRANA J/AU
L25          117 S E3-E9
                 E THOMSEN G/AU
L26           35 S E3-E6
L27            9 S L25,L26 AND L5
L28           25 S L24,L27
L29           18 S L5 AND (PD<=19990611 OR PRD<=19990611 OR AD<=19990611)
L30            2 S L28 AND L29
L31            0 S L5 AND (PY<=1999 OR PRY<=1999 OR AY<=1999) NOT L29
L32            9 S L27,L30
L33           16 S L29 NOT L32
                 SEL DN AN 2-9 11-16
L34            2 S L33 NOT E1-E38
L35           11 S L32,L34
L36            3 S L3 AND L29
L37           11 S L35,L36

     FILE 'HCAPLUS' ENTERED AT 09:57:27 ON 21 SEP 2004

     FILE 'BIOSIS' ENTERED AT 09:57:45 ON 21 SEP 2004
                 E WRANA J/AU
L38          142 S E3-E8
                 E THOMSEN G/AU
L39           45 S E3,E5,E9,E10
L40           60 S ?SMURF?
L41            6 S L38,L39 AND L40
L42           50 S L38,L39 AND ?SMAD?
L43            5 S L41 AND L42
L44            1 S L41,L43 AND PY<=1999

     FILE 'BIOSIS' ENTERED AT 10:00:17 ON 21 SEP 2004

     FILE 'WPIX' ENTERED AT 10:00:51 ON 21 SEP 2004
L45            8 S L4/BIX
L46            7 S L45 NOT THREAD/TI

     FILE 'WPIX' ENTERED AT 10:01:58 ON 21 SEP 2004

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